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The Metabolic Effects of 4-Aminopteroylglutamic Acid in the Guinea-Pig*

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(Received 4 July 1950)

The announcement of the synthesis of pteroylglutamic acid by Angier, Boothe, Hutchings, Mowat, Semb, Stokstad, SubbaRow, Waller, Cosulich, Fahrenbach, Hultquist, Kuh, Northey, Seeger, Sickels & Smith (1945) and reports of its effectiveness in the treatment of the haematological changes of pernicious anaemia were followed by the production of numerous synthetic analogues and derivatives, some of which were shown to be of value in the treatment of leukaemia and allied disorders. The synthesis of a potent substance of this nature, 4-aminopteroylglutamic acid (aminopterin), was announced by Seeger, Smith & Hultquist (1947).

Attempts to produce anaemia in animals by inducing folic-acid deficiency in various ways have been reviewed by Girdwood (1950), who refers to the use of 4-aminopteroylglutamic acid for this purpose in mice, rats, chicks, dogs and guinea-pigs. The reports suggest that in animals other than the guinea-pig the substance produces anaemia and leucopenia, and that these changes can be reversed with difficulty by high dosage of pteroylglutamic acid. Minnich & Moore (1948) produced a hypoplastic anaemia and agranulocytosis in guinea-pigs using 4-aminopteroylglutamic acid, but these changes could not be prevented by the simultaneous administration of liver extract or of pteroylglutamic acid.

The main purpose of the present investigation was to explore further the possibility of reversing the haematological changes induced by 4-aminopteroylglutamic acid in the guinea-pig, and to assay the livers of the animals for their content of pteroylglutamic acid and of vitamin B₁₂.

EXPERIMENTAL

Methods employed. Various tests were carried out on groups of at least five male guinea-pigs of about equal weight. Each test was controlled by the use of a group of untreated animals. All animals, including the controls, received 20 mg. ascorbic acid daily in solution, given by intraperitoneal injection. When 4-aminopteroylglutamic acid was administered it was given by intraperitoneal injection. All other injections were given intraperitoneally, with the exception of injections of *p*-aminobenzoic acid which were given into the subcutaneous tissues at the back of the neck. The animals were kept in separate cages with wire-grid bottoms, and were given unlimited amounts of a commercial diet containing a negligible quantity of folic acid.

* This work was carried out at the Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan, Ann Arbor, Michigan, during the period of tenure of a Rockefeller Travelling Research Fellowship.

Haematological observations. Cardiac puncture was used to obtain 0.3 ml. of blood, which was then placed in a small tube containing a measured amount of a dry mixture of ammonium and potassium oxalate. White blood-cell counts and red blood-cell counts were done in duplicate, standardized pipettes being used. Haemoglobin estimations were carried out in a Klett-Summerson photoelectric instrument. Haematocrit determinations were made in Van Allen tubes, which had been centrifuged at 3000 r.p.m. for 30 min. Peripheral blood examinations were carried out on cover-slip films that had been stained with Wright's stain. Differential counts were made on 100 cells. No bone-marrow studies were carried out on living animals, but, when the animal died or had been killed with ether, cover-slip films of the marrow were made and stained with Wright's stain.

Vitamin assays. Assays of pteroylglutamic acid were carried out by the method of Teply & Elvehjem (1945), using *Streptococcus faecalis* R. as the test organism and measuring the growth of the organism by turbidity estimations. The livers of the animals were homogenized in a Waring Blendor in a phosphate buffer mixture without enzyme preparations at pH 7.5. The homogenized material was then incubated for 24 hr. and kept in a refrigerator at -10° until tested. Repeat assays showed that storage in this way did not affect the amount in the samples either of growth factors for *Strep. faecalis* R. or for *Lactobacillus leichmannii*. Watery extracts of stools were used for assays of pteroylglutamic acid and vitamin B₁₂.

Vitamin B₁₂ assays were done by a modification of the method of Skeggs, Huff, Wright & Bosshardt (1948), using *Lb. leichmannii* 313 as the test organism. This test is less satisfactory than that for pteroylglutamic acid in that vitamin B₁₂ is only one of several factors that can replace each other in the growth of the organism (Kitay, McNutt & Snell, 1949; Shaw, 1949). The time of autoclaving was always exactly 7 min., and the pressure 15 lb. There was a gap of 24 hr. between the last injection of 4-aminopteroylglutamic acid, or other substances, and the killing of animals for these assay procedures.

RESULTS

The production of anaemia with 4-aminopteroylglutamic acid. Failure of attempts to prevent or reverse its action by administration of folic acid, liver, thymine or p-aminobenzoic acid

Effects of 4-aminopteroylglutamic acid alone

Fifteen animals of about 550 g. weight received 0.25 mg. 4-aminopteroylglutamic acid daily for 4 weeks. Anaemia was evident at the end of the 1st week, and after 4 weeks the mean red-cell count had fallen from 5,320,000 to 4,070,000/cu.mm. The haemoglobin level fell from 14.1 to 11.1 g./100 ml., and the mean corpuscular volume rose from 84.6 to 92.2 cu. μ . Leucopenia and thrombocytopenia did not develop. A low, continued reticulocytosis was seen (usually 2-7 %); this commenced about the 3rd week and was accompanied by the appearance of a few normoblasts in the peripheral blood.

When these animals were killed, the bone marrow was found to be cellular. It contained an increase of haemocytoblasts, proerythroblasts and early normoblasts,

but no cells that appeared to be comparable to the early, intermediate or late megaloblasts in man. As in experiments described by Innes, Innes & Moore (1949), an increase of reticulum cells was seen, and early forms of the white-cell series were numerous. The general condition of the animals remained good, and no marked naked eye or microscopic changes were found in the viscera.

A further five animals of 431 g. mean weight were given 0.25 mg. 4-aminopteroylglutamic acid daily for 7 weeks. The anaemia continued and, although it varied in degree from week to week, the mean red-cell count at 7 weeks was 4,280,000/cu.mm. and at no time was it less than 3,900,000/cu.mm.

Effects of various supplements

Six groups of guinea-pigs of about 550 g. weight received 0.25 mg. 4-aminopteroylglutamic acid daily with the following supplements.

Pteroylglutamic acid. Ten animals were given daily intraperitoneally 2.5 mg., and five 5.0 mg. pteroylglutamic acid.

Thymine. Five animals received approximately 0.45 g. thymine daily, some of it by mouth, some by means of a stomach tube, and the rest of it as an addition to the food.

p-Aminobenzoic acid. Five animals were given daily subcutaneously 2 ml. of a 10 % solution of a mixed sodium and potassium salt of *p*-aminobenzoic acid and had the same salt added as a 2 % supplement to the diet.

Liver injections. Five animals received daily intraperitoneally 4 U.S.P. units of a crude liver extract.

Liver by mouth. Five animals had a liver preparation in powder form added to the diet in the proportion of 5 %.

As compared with guinea-pigs receiving 4-aminopteroylglutamic acid alone, none of these groups of animals showed any significant alteration in the extent of the anaemia, in the bone-marrow changes or in general condition.

Effects of increasing the amounts of 4-aminopteroylglutamic acid and of pteroylglutamic acid

Five animals of 518 g. mean weight were given daily 0.5 mg. 4-aminopteroylglutamic acid and also 5 mg. pteroylglutamic acid intraperitoneally. These guinea-pigs became severely anaemic, the mean red-cell count falling at the end of the 5th week from 5,410,000 to 2,770,000/cu.mm. and the haemoglobin from 13.2 to 7.6 g./100 ml. The animals became lethargic and their hair roughened, but growth continued, although less satisfactorily than in the controls. The polymorphonuclear leucocyte count fell from 4950 to 630/cu.mm. at the end of the 1st week, but rose again to 1250/cu.mm. at the end of 5 weeks.

Five animals that received daily 50 mg. pteroylglutamic acid together with 0.5 mg. 4-aminopteroylglutamic acid, however, became very ill. They rapidly became emaciated, lethargic, and dehydrated. Four of the animals died, at 9, 11, 14 and 21 days, respectively, after the commencement of the injections. The urines contained much

albumin, but no crystals. At autopsy the livers showed marked passive congestion and degenerative fatty infiltration. The renal tubules of three animals contained eosinophilic material infiltrated with polymorphonuclear leucocytes. The bone marrow was cellular and did not differ from that of the animals previously described. Reticulocytosis did not occur.

Little stress can be laid on the blood counts in these guinea-pigs, owing to their very bad general condition and the fact that they became unable to drink water or to eat.

Four animals were given 50 mg. pteroylglutamic acid alone by injection. Some loss of weight occurred, but the animals did not become severely ill like those just described.

Effects of succinylsulphathiazole in addition to 4-aminopteroylglutamic acid

Further experiments were carried out to assess the effect of adding succinylsulphathiazole in powder form to the diet in the proportion of 2 %.

Succinylsulphathiazole alone was administered to eight animals for a period of 5 weeks and did not produce anaemia or other adverse effect.

Five animals were made anaemic by giving 0.25 mg. 4-aminopteroylglutamic acid daily for 5 weeks and, when there appeared to be some recovery from this anaemia, succinylsulphathiazole (2 %) was added to the diet. The reason for the recovery is unexplained, but the phenomenon has been noted by several workers using guinea-pigs. As will be seen from Table 1, the anaemia became more severe.

Table 1. *Effects on blood picture of adding 2 % succinylsulphathiazole to the diet of guinea-pigs already receiving 0.25 mg. 4-aminopteroylglutamic acid daily*

Time (weeks)	Weight (g.)*	Haemo- globin (g./100 ml.)	Red blood cells (millions/ cu.mm.)	White blood cells (/cu.mm.)	Polymorphs (/cu.mm.)	Packed cell volume (%)	Mean corpuscular volume (cu.μ.)
4-Aminopteroylglutamic acid given alone							
0	576 (5)	13.0	5.04	6030	1780	41.8	83.1
1	581 (5)	12.1	4.30	5730	1780	37.9	88.5
2	590 (5)	11.1	4.06	5670	1910	36.8	91.6
3	555 (5)	10.7	3.96	4010	1240	35.1	89.3
4	540 (5)	8.7	3.32	2450	560	30.2	91.6
5	543 (5)	9.8	3.51	4310	1350	31.3	90.4
Succinylsulphathiazole added to diet							
6	497 (5)	8.4	3.17	2810	1150	28.8	91.8
7	484 (4)	7.5	2.46	5180	2260	26.0	106.8
8	590 (3)	7.1	2.45	8530	4280	26.8	108.6
9	599 (3)	7.5	2.85	7010	2730	28.0	100.8
10	627 (3)	8.1	3.23	7350	2930	30.8	97.1
11	629 (3)	8.6	3.38	6280	3600	31.2	96.2
12	645 (3)	6.9	2.40	3820	1330	25.6	109.6

* Figures in parentheses indicate the number of animals alive at this time.

It is unfortunate that the animals selected for this study had lost weight before succinylsulphathiazole had been added to the diet, but they appeared to be recovering from this weight loss before the addition was made. The animals did not appear to be

unhealthy and there was no evidence of intestinal or other infection. Accordingly, the experiment was continued as planned.

Within a few days of the addition of the succinylsulphathiazole the animals, which had previously appeared to be healthy, became lethargic, refused to eat, and began to lose weight rapidly. The hair became rough and marked generalized oedema developed. There was individual variation in the response, however. One died in 7 days and another in 14 days.

Severe anaemia developed in all five animals, the red-cell count falling below 2,000,000/cu.mm. at some stage in the three animals that survived the experiment. Two animals then showed definite haematological improvement, but this was not maintained. The three animals that survived the first general effects of the addition of succinylsulphathiazole to their diet, while the administration of 4-aminopteroylglutamic acid was continued, then showed marked improvement, becoming active again, eating well, gaining weight, and looking like normal animals. One such animal had a fall in the red-cell count to 790,000/cu.mm., but only the pallor of the mucous membranes indicated that the animal was abnormal. This was in marked contrast to the condition of animals given larger doses of 4-aminopteroylglutamic acid in an effort to produce severe anaemia with it alone. There was no obvious relationship between the blood-cell counts and the general condition. The serum was not bile stained even when the anaemia was severe.

Improvement in the blood counts of these animals, when it occurred, was temporary, as has already been stated. Such improvement was preceded by a reticulocytosis and the appearance of normoblasts in the peripheral blood. Before such a development, the blood of these guinea-pigs differed from that of the previous groups in that a reticulocytosis did not accompany the anaemia. In one animal, the red-cell count was 1,815,000/cu.mm., and the haemoglobin 5.1 g./100 ml. 28 days after the commencement of the administration of succinylsulphathiazole. The reticulocyte count suddenly rose at this point from 0.4 to 38 %, and normoblasts appeared to the extent of 165/100 white cells. The serum was not bile stained. Seven days later, the red-cell count was 3,170,000/cu.mm., and the haemoglobin 6.9 g./100 ml. When the anaemia was most severe in all these animals, the polymorphonuclear leucocytes showed signs of toxic action. It will be seen that the mean corpuscular volume reached a higher value in this group than in any other animals.

Examination after death, or after the animals had been killed, showed that the marrows were cellular and contained an increase in megakaryocytes. Haemocyto blasts, proerythroblasts and early normoblasts were again seen. In addition, however, the most anaemic animal had cells that did not appear to belong to the normal red-cell series but showed some similarity to the early, intermediate and late megaloblasts found in man. The cells resembling the intermediate and late megaloblasts were very few in number. The former cells had a more open-work nucleus than the intermediate normoblast in the normal animal, but this nucleus was less oval than in the intermediate megaloblast in man. Late normoblasts were also very few, but red cells in the early stages of development were very numerous. Myeloblasts, too, were increased in number, and there were very many reticulum cells.

Protection by pteroylglutamic acid

This last experiment was repeated, but 2.5 mg. pteroylglutamic acid were given daily intraperitoneally in addition to 0.25 mg. 4-aminopteroylglutamic acid.

It will be seen from Table 2 that worsening of the anaemia did not occur when succinylsulphathiazole was added to the diet of this group of guinea-pigs. The general appearance of the animals at the end of the experiment was similar to that of guinea-pigs that received only 0.25 mg. 4-aminopteroylglutamic acid daily without succinylsulphathiazole.

Table 2. *Effects on blood picture of adding 2 % succinylsulphathiazole to the diet of five guinea-pigs already receiving 0.25 mg. 4-aminopteroylglutamic acid and 2.5 mg. pteroylglutamic acid daily. (The administration of the last two was continued throughout)*

Time (weeks)	Weight (g.)	Haemo- globin (g./100 ml.)	Red blood cells (millions/ cu.mm.)	White blood cells (/cu.mm.)	Polymorphs (/cu.mm.)	Packed cell volume (%)	Mean corpuscular volume (cu.μ.)
4-Aminopteroylglutamic acid and pteroylglutamic acid given							
0	426	13.4	5.28	8360	4620	46.0	87.6
1	443	11.3	4.43	6960	3880	39.3	91.0
2	476	11.6	4.24	5370	2760	39.1	95.4
3	494	10.7	4.27	5440	2510	37.9	89.6
4	536	10.3	3.87	4190	1400	33.6	86.8
5	566	10.1	4.26	7160	3710	35.4	83.6
Succinylsulphathiazole added to diet							
6	544	9.7	3.95	4840	940	33.7	85.6
7	589	10.1	3.99	3670	1240	31.9	80.6
8	608	10.0	4.08	5910	2220	32.5	80.0

In a further experiment, summarized in Table 3, two groups of five animals were given 0.25 mg. 4-aminopteroylglutamic acid and 2 % succinylsulphathiazole in the diet from the commencement. One of the groups received, in addition, 5 mg. pteroylglutamic acid daily. Again the effect of succinylsulphathiazole in increasing the anaemia was overcome by pteroylglutamic acid. At the end of 7 weeks the magnitude and character of the anaemia was similar to that in animals given 4-aminopteroylglutamic acid alone.

As before, there were individual variations in response to administration of succinylsulphathiazole and 4-aminopteroylglutamic acid alone. At the end of 7 weeks, three animals with low reticulocyte counts had red-cell values of less than 1,500,000/cu.mm., whereas another guinea-pig of similar weight had a count of 4,205,000/cu.mm. associated with a reticulocytosis of 23.5 %. The general features and bone-marrow changes were as before. The two most anaemic animals received daily by injection 5 mg. pteroylglutamic acid at the end of the 7th week. This was followed by reticulocytosis and a rise in the red-cell count.

Pteroylglutamic acid and vitamin B₁₂ content of the livers and stools

The results are shown in Tables 4 and 5. The results of the vitamin B₁₂ estimation showed a wide scatter, but the livers of the control animals, and of those receiving

Table 3. *Effects on blood picture of administration to groups of five guinea-pigs of 0.25 mg. 4-aminopteroylglutamic acid daily together with succinylsulphathiazole with and without pteroylglutamic acid*

Time (weeks)	Weight (g.)	Haemo-globin (g./100 ml.)	Red blood cells (millions/cu.mm.)	White blood cells (/cu.mm.)	Polymorphs (/cu.mm.)	Packed cell volume (%)	Mean corpuscular volume (cu.μ.)
4-Aminopteroylglutamic acid given							
0	408	13.5	5.32	8920	5030	46.3	87.3
1	432	12.9	4.61	7500	4230	38.8	84.8
2	446	9.1	4.26	5860	2620	39.1	92.2
3	474	10.9	4.21	5550	3100	36.8	91.1
4	487	10.4	4.24	7230	2100	37.1	88.5
5	518	11.2	4.51	8430	4500	39.3	80.7
6	522	10.2	3.72	4710	2530	33.2	88.7
7	529	9.9	4.14	4880	2870	35.3	86.1
4-Aminopteroylglutamic acid and succinylsulphathiazole given							
0	385	12.2	4.95	6970	3550	44.3	89.7
1	408	12.0	4.52	5640	2870	40.9	91.1
2	393	11.3	4.38	5840	1750	38.0	87.0
3	410	8.9	3.15	5860	2060	30.8	100.6
4	435	8.3	3.01	4420	1440	26.6	89.8
5	451	7.0	2.49	3960	600	23.5	95.7
6	473	6.8	2.34	3010	890	23.3	100.2
7	462	6.4	2.10	2450	490	21.3	101.6
4-Aminopteroylglutamic acid, pteroylglutamic acid and succinylsulphathiazole given							
0	385	12.7	5.11	5900	2340	42.9	84.1
1	389	11.2	4.28	4330	2060	37.9	89.9
2	395	10.6	3.92	5670	1830	35.0	89.7
3	399	10.1	3.67	4350	1170	33.9	93.7
4	408	10.1	3.44	6870	3100	32.4	95.1
5	437	9.5	3.43	4480	1150	32.1	95.1
6	469	9.7	3.51	5160	1760	33.6	96.9
7	486	9.1	3.78	5260	1650	31.9	82.5

Table 4. *Liver content of growth factors for Strep. faecalis and Lb. leichmannii in various groups of guinea-pigs*

Group	No. of animals	Strep. faecalis growth factors as pteroylglutamic acid (μg./g.)		Lb. leichmannii growth factors as vitamin B ₁₂ (μg./g.)	
		Mean	Range	Mean	Range
Normal controls	5	8.0	(6.8-11.5)	0.16	(0.08-0.31)
Receiving 0.25 mg. 4-aminopteroylglutamic acid daily	10	8.4	(4.8-12.5)	0.34	(0.14-0.76)
Receiving 0.25 mg. 4-aminopteroylglutamic acid and 0.45 gm. thymine daily	5	16.0	(4.2-26.7)	0.34	(0.24-0.43)
Receiving 0.25 mg. 4-aminopteroylglutamic acid and 2.5 mg. pteroylglutamic acid daily	5	18.6	(13.5-28.6)	0.49	(0.25-0.77)
Receiving 0.25 mg. 4-aminopteroylglutamic acid and 2 % succinylsulphathiazole daily	5	18.8	(13.6-26.5)	0.33	(0.19-0.48)

Table 5. *Stool content of growth factors for Strep. faecalis and Lb. leichmannii in various groups of guinea-pigs*

Group	No. of animals	Strep. faecalis growth factors as pteroyl-glutamic acid ($\mu\text{g./g.}$)		Lb. leichmannii growth factors as vitamin B ₁₂ ($\mu\text{g./g.}$)	
		Mean	Range	Mean	Range
Normal controls	4	13.9	(10.5-15.8)	0.44	(0.41-0.47)
Receiving 0.25 mg. 4-aminopteroyl-glutamic acid daily	4	10.7	(7.0-12.0)	0.60	(0.48-0.70)
Receiving 0.5 mg. 4-aminopteroyl-glutamic acid and succinylsulphathiazole daily	4	3.9	(3.0-4.9)	0.54	(0.45-0.61)

4-aminopteroylglutamic acid, contained less pteroylglutamic acid than the livers of the animals that had also been given pteroylglutamic acid, thymine, or succinylsulphathiazole.

As might be expected, this last group of guinea-pigs had less pteroylglutamic acid in the stools than the controls.

DISCUSSION

In guinea-pigs of suitable weight 0.25 mg. 4-aminopteroylglutamic acid daily caused moderate anaemia of macrocytic type with a cellular marrow that showed an increase in early red-cell types. The effects were not prevented by pteroylglutamic acid, thymine, *p*-aminobenzoic acid, or by liver preparations employed in the manner described.

The giving of succinylsulphathiazole in addition to 4-aminopteroylglutamic acid caused a more severe macrocytic anaemia with leucopenia and the appearance of cells in the marrow not unlike those seen in pernicious anaemia of man. Moreover, it was possible by this means to produce very severe anaemia in some of the animals without the development of toxic manifestations. This aggravation of the anaemia was prevented by giving pteroylglutamic acid, the degree of anaemia then being similar to that seen in the guinea-pigs given 4-aminopteroylglutamic acid without succinylsulphathiazole.

Other workers, using different animals, have shown that large amounts of pteroylglutamic acid are required to reverse the anaemia-producing effects of 4-aminopteroylglutamic acid (Seeger *et al.* 1947; Franklin, Stokstad & Jukes, 1948). It is of interest that in the present experiment death occurred when 50 mg. pteroylglutamic acid were given to a group of guinea-pigs in addition to 0.5 mg. 4-aminopteroylglutamic acid. It may be that it is not possible to administer sufficient pteroylglutamic acid to guinea-pigs to prevent the effects of 4-aminopteroylglutamic acid. There is also the possibility that 4-aminopteroylglutamic acid has, in this animal, an effect that is not due to its action as a folic-acid antagonist.

It is easy to speculate on the reason for the partial reversal of the effects of 4-aminopteroylglutamic acid given with succinylsulphathiazole by means of pteroylglutamic acid. Such speculation would serve little purpose while we lack sufficient information about the function of the *Leuconostoc citrovorum* factor (Sauberlich & Baumann, 1948; Nichol & Welch, 1950). It is usually thought that succinylsulphathiazole acts by killing bacteria able to synthesize pteroylglutamic acid. Reports on the ability of

certain micro-organisms to carry out such a synthesis have been given by Hutchings, Bohonos & Peterson (1941), Mitchell & Isbell (1942) and Thompson (1942).

In this instance, however, certain findings are not easily explained on the assumption that the effect of succinylsulphathiazole was due to such an action on intestinal bacteria, and that 4-aminopteroylglutamic acid and succinylsulphathiazole act by producing folic-acid deficiency in different ways. It has been shown by Minnich & Moore (1948) that large doses of 4-aminopteroylglutamic acid given for a relatively long period will produce anaemia. When this drug alone is used to produce severe anaemia, however, the marrow is hypoplastic, not cellular as in the present series of experiments. Moreover, as we have seen, it is possible to produce severe anaemia using 4-aminopteroylglutamic acid and succinylsulphathiazole without inducing general toxic effects, a result not found when 4-aminopteroylglutamic acid alone is used. The application of this observation to the treatment of leukaemia in man is at present being investigated.

It is not surprising that the liver content of pteroylglutamic acid was raised in animals receiving pteroylglutamic acid, or thymine which is also a growth factor for *Strep. faecalis*. On the other hand, the rise in the liver content of pteroylglutamic acid following the addition of succinylsulphathiazole to the diet is not easily explained. Further experiments of this nature, with larger groups of animals, are required.

We have seen that 5 mg. pteroylglutamic acid will not counteract the anaemia-producing effect of 0.25 mg. of the antagonist. Yet, since the animals in this experiment passed approximately 2.75 g. of stool in 24 hr., the controls passed 0.038 mg. of pteroylglutamic acid in this time, and succinylsulphathiazole eliminated 0.027 mg. from the stool over the same period. If the stool content of pteroylglutamic acid gives any indication of the amount being absorbed from the alimentary tract, it is surprising that amounts of this magnitude can so effectively prevent 0.25 mg. 4-aminopteroylglutamic acid from lowering the red-cell level below 4,000,000/cu.mm.

SUMMARY

1. Small groups of male guinea-pigs of about equal weight developed a mild macrocytic anaemia when they were given 0.25 mg. 4-aminopteroylglutamic acid daily. This action was not prevented by simultaneous administration of pteroylglutamic acid in quantities ten or twenty times as great, or by giving thymine, *p*-aminobenzoic acid or liver by mouth or injection.

2. The addition of succinylsulphathiazole to the diet in the proportion of 2 % resulted in a more severe form of macrocytic anaemia when 4-aminopteroylglutamic acid was given simultaneously in the above dosage. This aggravation of the anaemia was prevented by giving 2.5 mg. pteroylglutamic acid daily.

3. The guinea-pigs receiving pteroylglutamic acid, thymine or succinylsulphathiazole in addition to 4-aminopteroylglutamic acid had a higher liver content of pteroylglutamic acid than those receiving 4-aminopteroylglutamic acid alone. The stools of the animals receiving succinylsulphathiazole had a lower content of pteroylglutamic acid.

4. Reasons are given for doubting that 4-aminopteroylglutamic acid and succinylsulphathiazole act merely by producing pteroylglutamic-acid deficiency in different ways.

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The Nutrition of the Young Ayrshire Calf

1. The Endogenous Nitrogen and Basal Energy Metabolism of the Calf

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The concept of an endogenous nitrogen metabolism characterized by the urinary N excretion on a diet free of N (Folin, 1905) has been criticized by Schoenheimer & Rittenberg (1940) on the basis of their demonstration, in experiments on the fate of labelled amino groups in the animal body, of the extraordinary lability of tissue N. Burroughs, Burroughs & Mitchell (1940) and Mitchell (1948), however, have criticized the conclusion of Schoenheimer that no distinction is possible between the endogenous and exogenous metabolism of protein and have suggested that the endogenous metabolism of Folin represents the summation of those irreversible reactions involving N, exemplified by the reaction $\text{creatine} \rightarrow \text{creatinine}$. Whatever interpretation of this N excretion is made, the determination of the endogenous N metabolism remains a measure of a basal and inevitable loss of N from the animal body.

Palmer, Means & Gamble (1914) showed that the creatinine N excreted daily by men and women was proportional to their basal metabolic rates; in men the ratio, creatinine N (mg.):basal Calories of heat production, was 1.0 and in women 0.79. Terroine & Sorg-Matter (1927) extended these observations and showed that the ratio of total urinary N plus faecal N excretion (in mg.) on N-free diets to Calories of basal metabolism ranged from 2.2 to 2.6. This relationship was confirmed by Smuts (1935) who also showed that in mature animals varying in size from mice to pigs the ratio of endogenous urinary N to basal metabolism was 2.0 mg./Cal. In adult man much lower values have been obtained (Bricker, Mitchell & Kinsman, 1945; Murlin, Edwards, Hawley & Clark, 1946), some as low as 1.4 mg./basal Cal.

In the young animal, the basal metabolism/kg. body-weight is approximately two to three times that at maturity. In the past it has been usual to express endogenous N metabolism in mg./kg. body-weight. It is logical to suppose that the endogenous N metabolism of the young animal expressed in this way would be considerably higher than that of the mature animal, provided the 'Terroine-Sorg-Matter law' applies during the growth period. Few studies have been made of the effect of age on this relationship. Treichler (1939) has shown that, in the rat, age has a marked effect on the ratio of endogenous N metabolism to basal metabolism, giving a maximum ratio in early maturity and low values for the young and mature animal.

There are very few estimates of the relationship available for farm animals, for in cattle the only data are those which may be computed from the results of experiments

conducted for other purposes. Relevant experiments have been summarized by Mitchell (1929), Brody (1930) and Kehar, Mukherjee & Sen (1943) and these compilations are summarized in Table 1. The basal metabolism figures in the table have

Table 1. *Published data relating to the urinary nitrogen excretion of cattle on nitrogen-low or nitrogen-free diets*

Author quoted	Weight of animal (kg.)	Urinary N (g./day)	Endogenous N (g./kg.)	Computed basal metabolism (Cal./kg.)	Ratio endogenous N: basal metabolism (mg./Cal.)
Steenbock, Nelson & Hart (1915)	145	6.48	0.045	25.7	1.75
Honcamp, Koudela & Muller (1923)	385	16.32	0.042	14.9	2.81
Hart, Humphrey & Morrison (1912)	177	6.33	0.036	23.4	1.54
Honcamp <i>et al.</i> (1923)	440	16.40	0.035	13.0	2.69
Buschmann (1907)	443	15.0	0.034	13.0	2.61
Hart <i>et al.</i> (1912)	168	5.03	0.030	23.4	1.28
Buschmann (1907)	485	14.0	0.029	12.7	2.28
Hutchinson & Morris (1936)	?	12.70, 20.91	0.039	13.0	2.93
Kehar <i>et al.</i> (1943)	255 437	5.00 8.42	0.020 0.019	— —	— —
Mean, ignoring Kehar <i>et al.</i> (1943)					2.24

been computed from estimates of basal metabolism based on the equations given by Brody (1945). The results of Kehar *et al.* (1943) have not been used in the computation of the average, since no estimates of basal metabolism are available for Haryana bullocks under Indian conditions. The mean value of 2.24 mg. N/basal Cal. is in substantial agreement with the Terroine-Sorg-Matter law. It will be noted, however, that the lowest values of the ratio are for the smaller and younger cattle. The studies of Swanson & Herman (1943) on the endogenous N metabolism of cattle involving sixteen determinations were analysed by us statistically. When taken together with Brody's estimates of basal metabolism the results shown in Table 2 were obtained, suggesting that the

Table 2. *The endogenous nitrogen metabolism of cattle computed from the equations of Swanson & Herman (1943) and of Brody (1945)*

Body-weight (kg.)	Endogenous nitrogen		Basal metabolism† (Cal.)	Ratio endogenous N: basal metabolism (mg./Cal.)
	(g.)*	(g./kg.)		
50	3.68	0.073	1671	2.20
100	4.92	0.049	2899	1.69
200	6.59	0.033	4532	1.45
400	8.81	0.022	6379	1.38

* Computed from equation $E = 0.712 m^{0.42}$ (Swanson & Herman, 1943), where E = endogenous N/day and m = body-weight in kg.

† Computed from equation $\frac{Qb}{m} = 28e^{-0.0045m} + 11.4$ (Brody, 1945), where m = body-weight in kg., Qb = basal metabolism Cal.

ratio is highest in the smallest animal. This is probably due to errors of extrapolation of Swanson & Herman's equation, but in any case there are no experimental data or reliable estimates available on the relationship for the really young bovine.

Experiments were, therefore, carried out in which both the endogenous N metabolism and the basal metabolism were estimated in calves. The primary object of the experiments was to obtain an estimate of the endogenous N metabolism of the calf to permit estimation of the protein requirements for maintenance and growth by the Mitchell (1929) factorial procedure as modified by Blaxter & Mitchell (1948). Subsidiary objects were to study the distribution of urinary N in specific nitrogen inanition, the faecal N excretion of the young calf and the digestibility of diets containing dried milk.

EXPERIMENTAL

Plan of experiment

The experiment was planned to include four periods as follows: (1) A preliminary period of 14 days when a medium-protein diet was given. (2) A nitrogen-free feeding period of 8 days. (3) A recovery or final period of 6 days on the same diet as used in the preliminary period. (4) A period of starvation of 24–36 hr. at the end of which the basal energy metabolism was determined.

Calves and their management

Details of the three Ayrshire bull calves used are shown in Table 3.

Table 3. *Details of experimental calves*

Calf no.	Age at commencement of experiment (days)	Initial weight (kg.)	Notes	Diet (l./day)
1	40	34.2	A thin, poorly nourished calf	3.6
2	5	27.6	Normal	2.8
3	5	33.2	Normal	3.4

Calf no. 1 had already been used as an experimental animal and had been given low-calorie diets before the present experiment; calves nos. 2 and 3 received colostrum and whole cow's milk before the experiment.

The calves were housed in a room in which the air temperature was maintained between 55 and 65° F. by electric heaters. The night temperature fell on several occasions, however, to 52° F. and on two occasions the day temperature reached 70° F. In general over the 24 hr. a temperature gradient of 8° F. occurred.

The calves were confined to metabolism stalls with wire-mesh floors, permitting the quantitative collection of urine. Faeces were collected in light-weight watertight rubber faeces-bags attached by straps of rubber tubing to a single girth band of light canvas. The calf's tail was kept outside the faeces bag by means of a tight-fitting hole at the top of the bag. Each bag was removed daily and was replaced by a clean one. Since it was impossible to remove the whole of the faeces from the bag without washing

it, the weight of the faeces was obtained by noting the difference between the weight of the empty and full bag. Samples of urine and faeces were taken daily. All analyses were made on samples of pooled urine and pooled faeces, each representing a 2-day period, the only exception being urinary total N, which was determined daily.

The animals were weighed to the nearest 100 g. at 2-day intervals.

Pulse rates were recorded twice daily on each day of the experiment.

Diets

The experimental diets used are shown in Table 4. Their composition was based on the experience of Wiese, Johnson, Mitchell & Nevens (1947) and of Aschaffenburg, Bartlett, Kon, Walker, Briggs, Cotchin & Lovell (1949). No roughage or bedding of

Table 4. *Composition of the experimental diets*

Constituent	Normal diet, diet no. 6	N-free diet, diet no. 7
Dried skim milk powder (g./l.)	77.6	Nil
Lard (g./l.)	35.9	38.7
Cod-liver oil (ml./l.)	3.3	3.3
Glucose (g./l.)	14.8	95.0
Mineral mixture no. 2* (g./l.)	1.3†	13.0
Yeast extract (ml./calf)	Nil	20
Dry matter (g./l.)	130	147
Energy value (Cal./l.) (calculated)	740	750
N (g./l.) (by analysis)	4.6-4.8 depending on sample	<0.1‡

* See Table 5.

† In this mineral mixture magnesium was supplied as the sulphate.

‡ Varied slightly from calf to calf, each calf receiving 35.6 mg. N daily in the yeast extract.

any kind was allowed. The normal diet, diet no. 6, was made by dissolving the dried skim milk and glucose in water at 40°, raising the temperature of the solution to 70° and homogenizing both the lard and cod-liver oil into it at high pressure. The minerals were then added and the 'milk' made to volume. Sufficient was made to last for 3 days, and was stored at 0-5° in an immersion cooler. The N-free diet, diet no. 7, was made by homogenizing the fat directly into the glucose solution at a temperature of 85°. With this diet emulsifying agents had to be used to give stable emulsions. After considerable trial on a small scale, sodium tauroglycocholate, cholesterol and a proprietary preparation of sodium alkyl sulphates were found to be suitable agents. The same efficiency of homogenization was found when lecithin was used as an emulsifying agent. The quantities used in each 4 gal. (18 l.) batch were 10 g. sodium tauroglycocholate, 5 g. cholesterol and 8 ml. of the sodium alkyl-sulphate solution. Failure to homogenize the diet has been shown to cause profuse diarrhoea in the young calf and a loss of hair (Bate, Dwight & Cannon, 1946). We have noted similar diarrhoea in calves given poorly emulsified diets.

The mineral mixture used is shown in Table 5. This was made to resemble the ash constituents of whole cow's milk, with the exception that the magnesium content was

Table 5. *Mineral mixture no. 2 used to supplement the diets of the calves*

Major constituents	Quantity (g.)	Minor constituents	Quantity (g.)
$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	200	$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	0.5
K_2HPO_4	350	ZnCl_2	0.5
CaCl_2	100	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.5
MgO	40	CoCl_2	0.1
$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	150	KI	1.0
NaCl	80	Iron citrate	20.0
CaCO_3	100	NaF	0.5
Citric acid	150		

Table 6. *Major elements supplied by the mineral mixture compared with those supplied by cow's milk*

	Calcium	Phosphorus	Sodium	Potassium	Chlorine	Magnesium	Citrate
Cow's whole milk* (mg./100 ml.)	120	100	50	150	110	12	150
Mineral mixture no. 2 (mg./1.38 g.)	122.4	110.3	50.2	156.8	111.8	24.3	148

* Typical values.

doubled to prevent hypomagnesaemic tetany, and the essential minor elements were included. A comparison of the minerals supplied with those present in whole milk is given in Table 6. The yeast extract used was prepared according to the method of Macrae, El Sadr & Sellers (1942).

The diets were given to each calf in the quantities shown in Table 3. On every occasion the diet was warmed to 37° by immersion in a hot water-bath.

The following analytical methods were used:

Urine: Total N (Kjeldahl, using copper and selenium as catalysts); urea (Van Slyke & Cullen, 1910); ammonia (Van Slyke & Cullen, 1910); creatine and creatinine (Folin, 1914); uric acid (Benedict & Franke, 1922); allantoin (Larson, 1931-2); glucose (Benedict, 1911).

Faeces: Total N (Kjeldahl); dry matter (direct drying at 100°); ash (incineration at 550°); fat and soaps (Saxon, 1914). Non-protein N was determined on trichloroacetic-acid or alcohol filtrates of fresh faeces.

Basal metabolism. The oxygen consumption and carbon-dioxide production of each calf were determined after it had fasted for 24 hr., using the apparatus and technique described by Blaxter & Howells (1951). Collection of expired air for periods of 30 min. was made when the calf was lying quietly.

RESULTS

Body-weight. Changes in body-weight were not regular from weighing to weighing, although the calves were always weighed at the same time of day and before feeding. Differences in contents of bladder and digestive tract could account for this variation. Table 7 summarizes the changes in body-weight during the three periods.

With the exception of calf no. 3 in the preliminary period, in the periods when the calves were given diet no. 6 they gained in weight. The failure of calf no. 3 to gain was

largely due to acute diarrhoea and subsequent treatment by reduction of food intake and realimentation over a period of 4 days. In the 6 days following recovery from diarrhoea the gain in weight of this calf was 200 g./day. During the N-free feeding period, weight was lost to a small extent by calves nos. 1 and 3, whereas calf no. 2 continued to gain, though at a reduced rate. The normality of the gains in weight of these calves may be judged from the data compiled by Brody (1945). In the 1st month after birth Ayrshire calves grow at the rate of about 17 lb./month, or 257 g./day; in the 2nd month the growth rate increases to 30 lb./month, or 450 g./day. On this basis the gains of all calves during the period when diet no. 6 was given were subnormal.

Table 7. *Changes in the body-weight of the calves (g./day)*

Period	Calf no. 1	Calf no. 2	Calf no. 3
Preliminary (diet no. 6)	+213	+219	-156
N-free (diet no. 7)	-58	+158	-33
Final (diet no. 6)	+211	+237	+237

Pulse rate and general behaviour. There was a slight rise in the pulse rates of the calves during the first few days on which the low-N diet was given. Increased rates were observed in all calves, but the daily variation was not sufficient to warrant a conclusion that the effect in each individual was significant. A general decline in pulse rate with progress of the experiment from initial values greater than 100 to values of 70-85 occurred. This appeared to be an age effect, probably related to a decline in metabolism with advancing age of the young animal. The pulse rate of calf no. 3 during the period of diarrhoea when its food intake was reduced dropped to 60-65/min. These data were not included in the statistical analyses.

Throughout the periods in which diet no. 6 was given, all calves behaved normally with the exception of calf no. 3, as noted above. In the N-free feeding period, however, difficulties arose in persuading the calves to eat the diet. This was not an immediate reaction. All calves consumed the diet without concern for the first 3 days. On the 4th there was some reluctance to drink, and this reluctance increased and continued throughout the remainder of the period. Similar disturbances of appetite on feeding low-N diets to sheep have been observed by Miller (1937) and by Ferguson & Neave (1943).

All calves had diarrhoea at times when given the N-free diet. This appeared to be the result of a dietetic disturbance rather than bacterial infection and was probably due to the calves' inability to homogenize their faecal excretion of fat into the fluid phase on this diet. Slight shivering of the calves was noted on several occasions. Its cause is not known, but it was not an indication of increased excitability since the calves were lethargic and dull throughout. Otherwise the calves behaved normally in every respect and, despite the diarrhoea, seemed content. At the end of the experiment the calves were slaughtered. Their rumens were quite undeveloped, though some growth had occurred. It was clear that no active fermentation of the diets had taken place in this organ.

Digestibility of the diets. Table 8 summarizes the data relating to faecal excretion of water and dry matter and the apparent digestibility of the ingested dry matter.

Although the day-to-day variation in faecal excretion was quite large, it is clear that large increases in the mean faecal excretion occurred when the calves were given the N-free diet. Analysis of variance of the results in Table 8 showed that statistically the increase in dry matter and water excretion was highly significant ($P < 0.001$), as was the fall in digestibility of the dry matter of the diet. The number of degrees of freedom for testing was, however, only two.

Table 8. *Mean daily excretion of water and dry matter in the faeces and digestibility of the dry matter ingested by the calves*

Calf no.	Preliminary period, diet no. 6	N-free period, diet no. 7	Final period, diet no. 6
Mean daily excretion of dry matter (g.)			
1	32.3	122.5	49.6
2	21.0	112.4	18.3
3	25.2	100.0	24.7
Mean	26.2	111.6	30.9
Mean daily excretion of water (g.)			
1	165.6	989.3	216.9
2	93.7	435.1	88.4
3	64.3	1366.2	87.5
Mean	107.9	930.2	130.9
Mean daily apparent digestibility of dietary dry matter (%)			
1	93.2	77.3	89.6
2	94.4	73.2	95.1
3	94.3	80.4	94.5
Mean	94.0	77.0	93.1

The unweighted mean composition of the faeces for each calf, computed from the separate determinations, is summarized in Table 9, and Table 10 summarizes the statistical significance of the changes in percentage composition.

Table 9. *Mean percentage composition of faecal dry matter of the calves*

Constituent	Calf no. 1		Calf no. 2		Calf no. 3	
	Pre-liminary period, diet no. 6	N-free period, diet no. 7	Pre-liminary period, diet no. 6	N-free period, diet no. 7	Pre-liminary period, diet no. 6	N-free period, diet no. 7
Dry matter in fresh faeces	17.6	11.4	20.1	20.6	19.5	6.8
Ash in dry matter	21.4	8.0	14.1	7.2	12.9	8.9
Total fat in dry matter	35.3	64.1	28.0	66.0	39.5	63.7
N × 6.25 in dry matter	37.1	12.6	58.4	10.2	47.2	12.7
Residual material, i.e. 'carbo-hydrate'	6.2*	15.2	−0.5	10.2	0.3	16.7
Fat present as soaps†	54.6	38.6	63.1	15.3	37.3	23.1
Fat present as neutral fat + free fatty acids + unsaponifiable residue†	45.4	61.4	36.9	84.7	62.7	76.9
N present as non-protein N‡	24.2	< 4	21.0	12.1	22.4	14.7

* This animal had access to sawdust bedding for 2 days before the preliminary period began. Some was eaten and small quantities appeared in the faeces during the first 4 days of the preliminary period. The value does not represent undigested dietary carbohydrate.
† Percentage of total fat.
‡ Percentage of total nitrogen.

Table 10. *Mean composition of the faeces of the calves and digestibility of the diet given in the preliminary period and in the nitrogen-free feeding period*

	Preliminary period, diet no. 6	N-free period, diet no. 7	Difference	Significance
Ash in dry faeces (%)	16.1	8.0	8.1 ± 2.76	N.S.
Fat in dry faeces (%)	34.3	64.6	30.3 ± 4.04	$P < 0.02$
N in dry faeces (%)	7.6	1.9	5.7 ± 1.10	$P < 0.05$
Residual 'carbohydrate' (%)	2.0	15.5	13.5 ± 2.38	$P < 0.01$
Fat excreted (g.)	10.5	74.9	64.4 ± 1.75	$P < 0.01$
Apparent digestibility of dietary fat (%)	91.7	44.9	46.8 ± 3.56	$P < 0.01$
Energy excreted in faeces (Cal./day)	172	844	672 ± 36.9	$P < 0.01$
'Apparent digestibility' of dietary energy (%)	92.9	66.5	26.4 ± 2.52	$P < 0.01$

N.S.: not significant.

It will be noted that large and statistically significant mean changes occurred in the fat content and the N content of the faeces when the N-free diet was given and that the increase in the residual fraction (presumably carbohydrate) was highly significant. In so far as the dry-matter excretion increased during the N-free feeding period, it is clear that there were marked changes in the total daily excretion of fat and in the digestibility of fat. These are shown in the lower part of Table 10.

Total fat excretion increased six times during the experimental period, and the digestibility of dietary fat dropped to less than half the normal value. In this respect, the digestibility data recorded by Schneider (1947), based on a study of the older German and Russian literature, suggest that the apparent digestibility for the calf of butterfat in cow's milk is 93–99, values only slightly higher than those we have found with our diets, containing dried skim milk with lard as a source of fat. The increase in dry-matter excretion, shown in Table 8, is thus largely due to a decrease in fat absorption, since 75 % of the increased faecal dry-matter excretion consisted of fat. This failure to digest fat is also shown by the lowered content of soaps in the faeces of the calves (see Table 9) and obviously implies a considerable reduction in the number of calories available to the animal. The faecal calories and the 'digestibility' of the ingested calories were calculated from the compositional data, using factors of 9.1 Cal./g. for fat, 4.0 for carbohydrate and 5.6 for protein ($N \times 6.25$). These results are also shown in Table 10 and emphasize the importance of this fall in digestibility of fat when maintenance of an adequate calorie intake is being considered.

The reason for such a fall in digestibility of fat on feeding diet no. 7 is not certain. The fat level remained fairly constant and the same fat was used throughout the experiment. This suggests, but does not prove, that a factor, or factors, in dried skim milk is essential for fat absorption. Whether this is the phosphoprotein of the dried skim milk or the small quantity of phospholipin present or yet another factor remains to be determined.

Nitrogen balance, metabolic faecal nitrogen and endogenous urinary nitrogen. The N-balance results are shown in Table 11. Statistical analysis of these results showed

that there was no significant change in faecal N excretion but a highly significant ($P < 0.01$) change in urinary N excretion and N balance when the N-free diet was given.

Table 11. *Nitrogen-balance results for the calves (g./day)*

Calf no.	Preliminary period, diet no. 6				Experimental period, N-free diet no. 7				Final period, diet no. 6			
	Excretion				Excretion				Excretion			
	Intake	Faeces	Urine	Balance	Intake	Faeces	Urine	Balance	Intake	Faeces	Urine	Balance
1	15.86	1.25	7.87	+6.74	0.04	2.45	2.99	-5.40	15.13	2.49	9.42	+3.22
2	12.34	2.02	6.69	+3.62	0.04	1.76	2.24	-3.97	11.77	1.15	5.90	+4.72
3	15.31	2.17	9.42	+3.72	0.04	2.08	2.52	-4.56	14.29	1.49	7.93	+4.87
Mean	14.50	1.81	7.99	+4.70	0.04	2.10	2.58	-4.64	13.73	1.71	7.75	+4.27

The faecal N excreted when a N-free diet is given represents the so-called metabolic N of the faeces, that is the inevitable drain of N from the tissues incidental to food ingestion and digestion. This has been shown in the rat to be proportional to the dry matter ingested (Schneider, 1934, 1935). In adult ruminants the metabolic faecal N is approximately 0.5 g./100 g. dry matter consumed (Blaxter & Mitchell, 1948) a value about five times as great as that observed in the rat or man. Since large quantities of fibre in the diet of rats increase the metabolic faecal N (Mitchell, 1926) this difference between ruminants and non-ruminants has been regarded as entirely due to the higher fibre content of the ruminant's diet. In these milk-fed calves, much lower values of metabolic N would, therefore, be expected than in mature ruminants. When, however, the N excretion in the N-free period is related to the dry-matter intake of these calves, values of 0.45, 0.42 and 0.41 g./100 g. dry matter ingested are obtained, values well within the range of those quoted by workers with adult ruminants. These results might suggest that the species differences in metabolic faecal N excretion quoted above are true species differences established at a very early age. A more probable explanation, however, is that the digestibility of the dry matter of the diet determines the metabolic faecal N excretion. This contention is supported by the fact that the N excretion of the calves was, on the average, higher during the N-free period than in the periods of normal feeding when digestibility of the total diet was high. The solubility and distribution of the N of the faeces produced on normal diets differed from that found when N-free diets were given. Mukherjee (1946), working with Indian bullocks, has similarly shown that the metabolic faecal N excretion is more closely related to dry-matter excretion than to dry-matter intake. Such an explanation would account for the high values found in these calves fed on diets free of fibre.

The urinary N in the N-free period represents the endogenous urinary N of the calf. The results are shown graphically in Fig. 1. It will be noted that the continuing metabolism of N (Thomas, 1909) in the calf is very small, constant levels of urinary N being reached within 2 days. The urinary N was much lower during the N-free period than the urinary N excreted during the period in which diet no. 6 was fed. The interpretation of this observation will be discussed later.

The magnitude of the N balances during the period of N inanition was approximately

related to the body size of the calves. During the period of diarrhoea in calf no. 3, the loss of N from the body was 13·5 g./day despite the fact that food intake was only reduced to 67 % of the normal over the period concerned. This figure, representing katabolism of body N, suggests that an adequate supply of energy was being ingested by the calves during the N-free period despite the low digestibility of their ration.

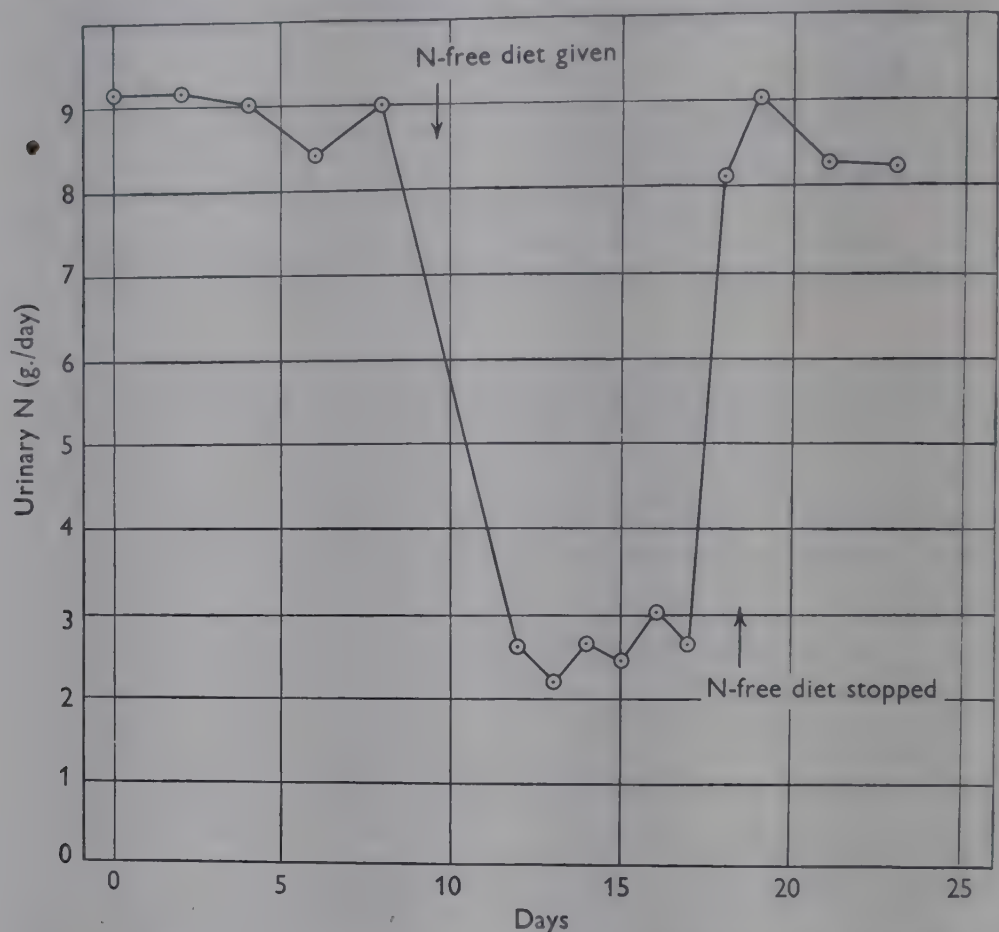


Fig. 1. Effect of a nitrogen-free diet on the urinary nitrogen of the calf.

Distribution of urinary nitrogen. The distribution of urinary N for each calf is summarized in Table 12. It will be noted that the data relating to allantoin N are not

Table 12. Mean daily excretion of nitrogen in different nitrogenous metabolites in the urine of the calves

Metabolite	Preliminary period, diet no. 6				Experimental period, N-free diet no. 7				Final period, diet no. 6				Statistical significance of change during N-free period
	Calf no. 1	Calf no. 2	Calf no. 3	Mean	Calf no. 1	Calf no. 2	Calf no. 3	Mean	Calf no. 1	Calf no. 2	Calf no. 3	Mean	
Urea (g.)	4·55	4·20	5·35	4·70	1·04	1·02	1·07	1·04	6·38	3·66	4·95	5·00	$P<0\cdot01$ $P<0\cdot05$ $P<0\cdot01$
Ammonia (g.)	0·98	0·68	1·82	1·16	0·34	0·21	0·46	0·34	1·12	0·76	0·65	0·84	
Total urea and ammonia (g.)	5·54	4·88	7·17	5·87	1·39	1·22	1·53	1·38	7·50	4·41	5·60	5·84	
Creatine (g.)	0·248	0·221	0·189	0·219	0·221	0·025	0·063	0·103	0·139	0·112	0·173	0·144	$P<0\cdot05$ N.S. N.S.
Creatinine (g.)	0·346	0·342	0·454	0·380	0·221	0·344	0·392	0·319	0·354	0·257	0·384	0·332	
Uric acid (g.)	0·050	0·029	0·054	0·045	0·048	0·028	0·047	0·041	0·069	0·026	0·086	0·060	
Allantoin (g.)	0·811	0·575	0·746	0·711	See text, this page.				See text, this page.				—

complete. The method of Larson (1931-2) was used to determine allantoin during the preliminary period, and when this method was applied to the urines excreted during the N-free period it gave values greatly in excess of expectation, necessitating con-

siderable dilution of the urine. The apparent increase was so great that the residual N became negative. Interference was traced to the relatively large quantities of reducing sugar excreted by the calves, which were not removed by the procedure of phosphotungstic-acid precipitation employed. The method was therefore abandoned. Later, stored samples representative of the N-free period were analysed by a slight modification of Young & Conway's (1942) method using the Rimini-Schryver reaction. Results were obtained which agreed with those obtained in the preliminary period using Larson's method. The urinary excretion of sugar during the low-N period was probably an alimentary glycosuria comparable to the lactosuria noted in calves by Rojas, Schweigert & Rupel (1948). The urinary concentration of sugar estimated by Benedict's (1911) method rose to over 2 %.

Urine volume declined during the low-N period and the specific gravity of the urine rose. This probably was a result of adaptation to the high loss of water in the faeces. Consequently the concentration of some of the metabolites in urine increased during the low-N period. The last column of Table 12 shows the statistical significance of the mean changes in N distribution in the period when the N-free diet was given. The fall in total N excretion was largely due to a fall in the excretion of urea and ammonia, with a smaller fall in creatine elimination. Creatinine excretion was not changed significantly nor was the excretion of uric acid. Although the data are not complete, the few analyses for allantoin did not indicate any fall in its excretion during the period of N-free feeding. Table 13 expresses the percentage distribution of the urinary metabolites. Uric acid, creatinine and residual N (largely allantoin) make up a far greater proportion of the total N during the N-free period than during protein feeding. The interpretation of this table is given in the summary.

Table 13. *Distribution of nitrogen in different nitrogenous metabolites in the urine of the calves, expressed as percentages of total urinary nitrogen*

Metabolite	Preliminary period, diet no. 6	Experimental period, diet no. 7	Final period, diet no. 6
Urea	58.8	37.8	64.4
Ammonia	14.5	12.1	10.8
Urea and ammonia	73.4	49.9	75.2
Creatinine	4.7	11.8	4.3
Creatine	2.7	3.9	1.9
Uric acid	0.6	1.5	0.7
Residual N	18.6	32.9*	17.9

* According to analyses by the method of Young & Conway (1942), over 50 % of this consists of allantoin.

Basal metabolism. The results of duplicate determinations are given in Table 14. The agreement between duplicate determinations was close and analysis of variance of heat production showed that the coefficient of variation was less than 4 % (three degrees of freedom). The metabolism of the calves was intense since, if Brody's (1945) estimates of the basal metabolism of the adult cow are correct, then their metabolism per kg. body-weight was three times that found at maturity.

Table 14. *Respiratory metabolism of the calves*

Variable measured	Calf no. 1		Calf no. 2		Calf no. 3	
	Determination no.		Determination no.		Determination no.	
	1	2	1	2	1	2
Environmental temperature (° F.)	73	72	68	68	64	61
Pulse rate/min.	59	55	57	56	52	53
Respiratory rate/min.	16.8	15.5	16.0	14.8	19.4	18.5
Minute volume of respiration (l.)	4.52	4.79	3.88	2.98	3.98	4.23
Tidal air (ml.)	269	309	243	201	205	229
Oxygen consumed (l./hr.)	13.17	12.69	10.70	10.27	13.06	12.96
Carbon dioxide produced (l./hr.)	9.80	9.43	7.95	7.21	9.67	9.13
R.Q.	0.74	0.74	0.74	0.71	0.74	0.71
Heat production (Cal./24 hr.)	1495	1440	1214	1155	1481	1457
Heat production (Cal./kg./24 hr.)	42.7	41.2	43.1	41.0	45.9	45.1

DISCUSSION

The metabolic faecal N excretion of the calves and the digestibility of the diets have already been adequately considered in the appropriate sections. This discussion deals only with the relation of endogenous N metabolism to basal metabolism. It is necessary in the first instance to assess the validity of the determinations of endogenous N and basal metabolism that have been presented. Too high an estimate of either quantity would result in many difficulties of interpretation of the ratio of the one to the other.

The prerequisites for a basal metabolism determination were probably maintained, the possible exception being that the critical temperature of the calf may be somewhat higher than the environmental temperature at which the determinations were made. This might be inferred from the results with calf no. 3 given in Table 14. This calf's metabolism was higher than that of the others and the environmental temperature was lower than had been maintained for calves nos. 1 and 2. Muscular repose was maintained, and the long periods of observation employed obviated errors due to any initial disturbance of the animals.

The endogenous N excretion was determined when the calves were ingesting a diet of low digestibility and when a further part of the dietary energy was lost as sugar in the urine. This low intake of energy probably accounted for the fall in the pulse rates of the calves after an initial rise when the diet had been given for a few days. Calculations of the metabolizable calories available to the animal from analytical data for urine and faeces gave in all instances values above the directly determined basal energy metabolism, and, provided that the heat increment of the diet was small—a reasonable assumption in an animal not converting a large portion of its dietary carbohydrate to lower fatty acids—these intakes of metabolizable energy should have been adequate to meet basal requirements. A further point is that in two calves creatine completely disappeared from the urine by the end of the period and in the other it reached a very low level. It would appear, therefore, that these nitrogen excretions were truly endogenous.

The endogenous N metabolism of the calves and their basal metabolism are shown in Table 15. For comparative purposes the two equations for basal metabolism and endogenous N metabolism estimation from body-weight for mature animals of different

Table 15. *Relation between the basal metabolism of the calves and their endogenous nitrogen metabolism*

Calf no.	Weight (kg.)	Endogenous N metabolism		Basal metabolism		Ratio mg. N:Cal.
		(g./day)	(mg./kg./day)	(Cal./day)	(Cal./kg./day)	
1	35.7	2.99	83.8	1467	41.9	2.00
2	27.8	2.24	80.8	1185	42.0	1.93
3	31.0	2.52	81.0	1469	45.5	1.78
Mean	31.5	—	81.9	—	43.1	1.90 ± 0.07
Expected values in mature animals (Brody, 1945)	31.5 8.0	— —	55.6 79.3	— —	28.1 43.1	— • —

species (Brody, 1945) have been used to calculate, firstly, the expected basal metabolism and endogenous N metabolism of a mature animal of the same mean body-weight as the calves and, secondly, the expected endogenous metabolism of a mature animal having the same basal metabolism per kg. body-weight (a much smaller animal of course) as the experimental calves. The results in Table 15 suggest that the endogenous N metabolism of the young calf is far more intense per kg. body-weight than that of a mature animal either of the same size or the same species (cf. Table 1). The same is true of the basal energy metabolism. The ratio of endogenous nitrogen to basal metabolism, the so-called Terroine-Sorg-Matter law, appears the same in the young calf as in mature animals of different species, namely about 2.0 mg. N/basal Cal. of heat production.

The percentage distribution of the nitrogenous metabolites in the urine of the calves on the N-free diet presented in Table 13 shows that some 11.8 % of the endogenous N can be accounted for by the irreversible reaction creatine → creatinine. A further 25 % or more is clearly the result of enzymic degradation of purine compounds of the body. Of the total, however, 50 % is due to an inevitable katabolism resulting in the end-products urea and ammonia. Whether this katabolism represents the destruction of tissue proteins to supply amino-acids, methyl groups, or other carbon fragments essential to the economy of the animal is not known. The percentage of creatinine N in the endogenous N excretion of these calves was lower than the percentage normally found in adult man. Brody (1945) has shown that in mature animals the percentage of the total endogenous N present as creatinine increases with body size, for creatinine excretion is related more to body mass than to body surface. The percentage to be expected in a mature animal with the same body-weight as the calves is 15.8. The ratio expected in a mature animal with the same basal metabolism, however, is 11.9, a value in very close agreement with the value of 11.8 found in these studies.

SUMMARY

1. Experiments with three young dairy calves are described in which the endogenous nitrogen and basal metabolism were determined.
2. The sole diet of the calves was a semi-synthetic liquid diet resembling milk. When protein was an ingredient of the diet, body-weight gains were slightly subnormal.

3. The apparent digestibility of the dry matter dropped from 94.0 to 77.0 % on giving a N-free diet. This was associated with a decrease from 91.7 to 44.9 % in the apparent digestibility of the dietary fat, and a decrease in the 'digestibility' of the calories of the diet from 92.9 to 66.5 %. The percentage of the total fat present as soaps fell when the N-free diet was given.

4. Nitrogen balances were +4.70 and +4.27 g./day in the periods in which a diet containing dried-milk protein was given. Negative balances of -4.64 g./day occurred when the N-free diet was given.

5. The N content of the faeces during the N-free feeding period was 0.427 ± 0.013 g./100 g. dry matter ingested. This result is discussed in relation to species differences in metabolic faecal N excretion, and it is suggested that the quantity of dry faeces excreted per day rather than dry-matter intake determines metabolic N excretion.

6. The endogenous urinary N was 81.9 mg./day/kg. body-weight. The basal energy metabolism was 43.1 Cal./kg./day. The amount of endogenous N excreted per Cal. of basal heat produced was 1.90 ± 0.068 . This value for these animals with their intense metabolism in relation to their body size is in close agreement with that found by Smuts (1935) for mature animals of different species.

7. The distribution of the urinary N changed on giving the N-free diet. Creatine excretion fell to a very low level; creatinine, uric acid and allantoin excretion remained constant, while urea and ammonia excretion increased.

8. The distribution of the urinary N when the N-free diet was given suggests that 12 % of the endogenous metabolism involves the irreversible reaction creatine $\rightarrow$ creatinine, 25 % or more involves purine katabolism, and 50 % involves reactions terminating in the excretion of urea and ammonia.

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The Nutrition of the Young Ayrshire Calf

2. A Spirometer for the Determination of the Respiratory Exchange of the Calf

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AGRICULTURAL RESEARCH COUNCIL)

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Determination of the energy exchange of farm animals by methods involving the principle of indirect calorimetry presents a number of technical difficulties. These are mostly due to the higher rate of lung ventilation in farm animals than in man. In cattle and sheep the minute volume of the respiration increases with increasing environmental temperature since in these species loss of water vapour from the respiratory passages is an extremely important channel of heat emission. With young cattle of the same weight as man, low environmental temperatures are associated with minute volumes of approximately 8–10 l., values quite comparable to those recorded for man, but at environmental temperatures approaching the animal's body temperature, values of over 25 l. may be obtained. Methods of indirect calorimetry involving the use of the Douglas bag or of the Tissot spirometer have never been popular in studies of energy metabolism in farm animals (Orr & Magee, 1923) since the large volumes of expired air soon fill the apparatus, and most of the work in this field has been conducted with large respiration chambers through which a measured stream of air is passed. Such instruments are expensive and cumbersome in use. Brody (1945), however, has

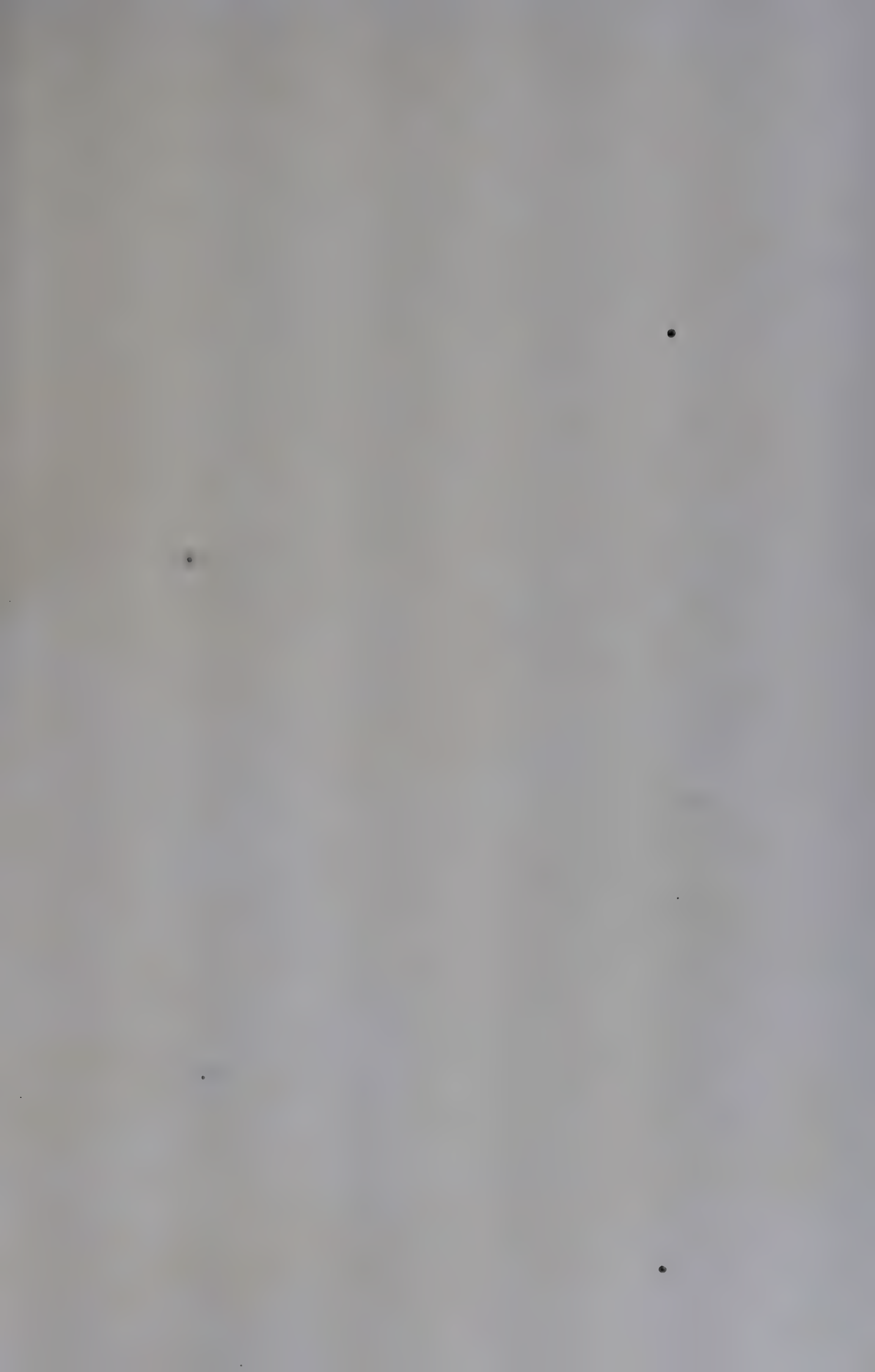
devised a closed circuit method for determining the oxygen consumption, and hence the energy exchange, of animals varying in size from lambs to large steers. This method has many advantages in adaptability and ease of use. It has also decided disadvantages. Firstly, no estimate of the respiratory quotient is obtained; secondly, the carbon-dioxide absorbent causes some considerable resistance to the animal's respiration; thirdly, methane and hydrogen accumulate in the bell giving an underestimation of the true oxygen consumption, and lastly, the periods of time during which the determination is made tend to be rather too short for maximal accuracy. The absence of an estimate of the carbon-dioxide production and hence of the R.Q. is an obvious disadvantage though the error involved is in fact small. Analysis of the residual gas in the bell gives a valid estimate of the methane and hydrogen error. It was thought, however, that direct analysis of expired air, collected and measured over long periods of time, would be a more reliable method to use in studies on calf nutrition, and for this reason a very large spirometer has been built making possible accurate determinations both of oxygen consumption and of carbon-dioxide production over periods of 30-45 min.

EXPERIMENTAL

Construction of spirometer

Fig. 1 shows an elevation of the spirometer from which the general dimensions can be obtained, and Pl. 1 shows the instrument with mask and rubber-tubing connexions attached. The base consists of an angle-iron and wood frame which can be levelled by means of levelling screws (*H*) at each corner. The frame (*N*) carrying the pulleys (*G*) is also constructed of angle iron, and, for added rigidity, is stayed to the top of the outer wall of the galvanized iron tank. The main assembly consists of a special water tank and a light bell. The former is made of two tanks, the inner one being closed at the top and completely sealed. This allows a water space of 2 in. between the walls of the inner and outer tank in which the bell is free to rise or fall. These main tanks are constructed of 20 s.w.g. iron and were galvanized after assembly to prevent rust. Two 1 in. bore pipes pass through the top of the inner tank and can be connected through low resistance taps (*A*, *B*) of 1 in. bore to one-way valves and a face mask. These pipes are fitted with drainage cocks (*L*) at their lowest level since water vapour from the air expired by the animals tends to condense in them. The bell (*C*) is constructed of 18 s.w.g. aluminium sheeting and is lightly wired at the base to ensure rigidity. The bell is suspended by a thin cord which passes over two pulleys (*G*) mounted on ball races and is attached to the top of a brass tube (*M*) screwed into the centre of the top of the bell. This tube acts as a guard for a thermometer and is, of course, air-tight.

The correct counterpoising of the spirometer bell presented some difficulties since in such a large instrument it was not practicable to adopt the usual principle of concentric pulleys to compensate for the apparent decrease in weight of the bell when immersed. Nor was it possible to use a heavy chain instead of a light cord to compensate for this decrease in weight. A modification of the rigid automatic siphon tube of Tissot's (1904) original instrument was therefore devised. This consisted of a flexible levelling device and has proved extremely successful. The counterpoise weight (*F*)





General view of the spirometer

was made from a copper tube and a glass tube, the internal cross-sectional area of which was calculated to be equivalent to the cross-sectional area of the aluminium metal of the

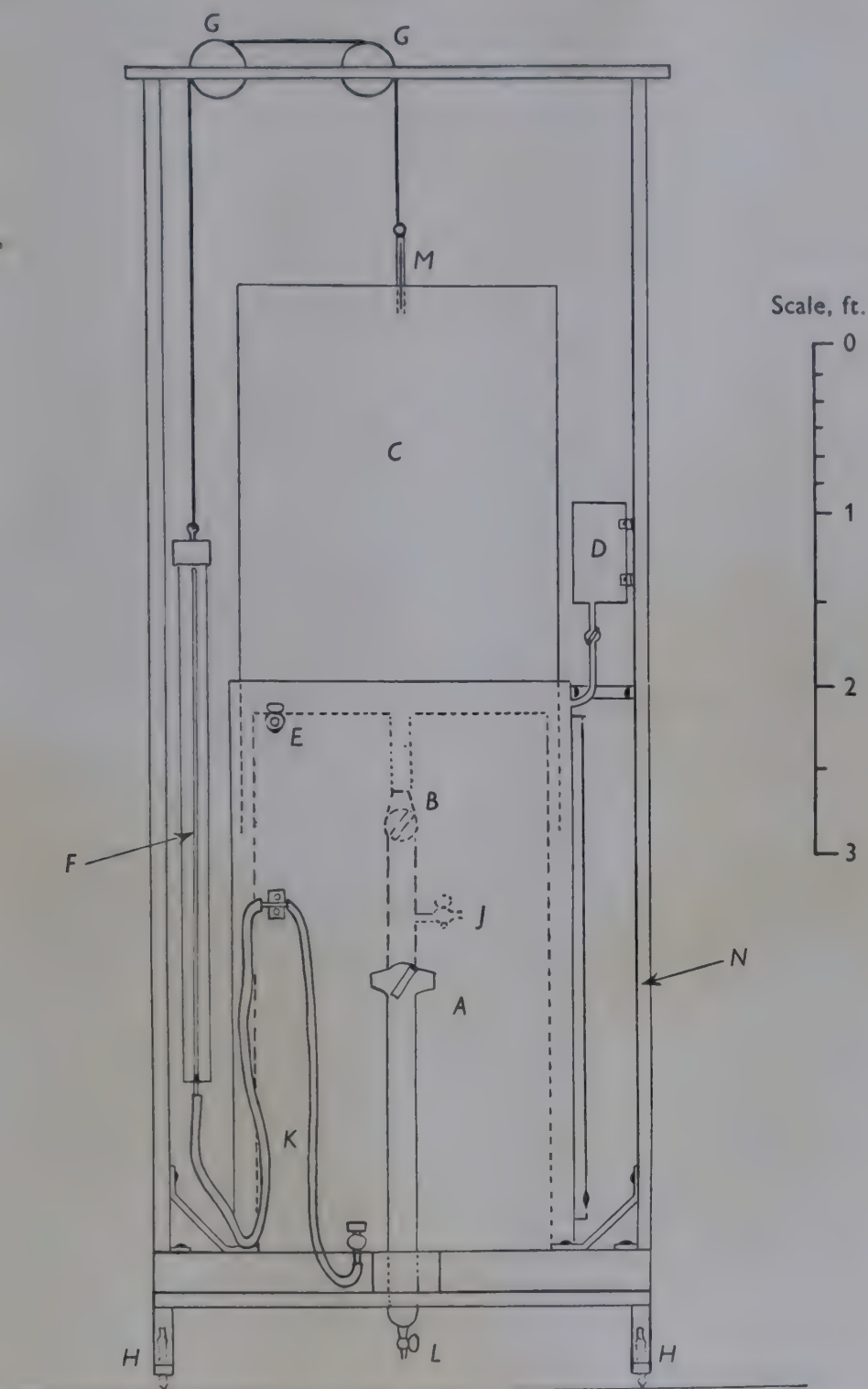


Fig. 1. Elevation of spirometer showing counterpoise device. *A*, ingoing-air two way stopcock and pipe; *B*, rinsing stopcock and pipe; *C*, aluminium bell; *D*, levelling device for water level in main tanks; *E*, overflow tap used in conjunction with *D* to maintain water level; *F*, counterpoise-siphon weight; *G*, ball-bearing pulleys; *H*, levelling screws; *J*, sampling stopcock; *K*, rubber-tubing siphon; *L*, drain cocks for inlet and rinsing tubes; *M*, thermometer and thermometer housing; *N*, framework.

bell multiplied by its specific gravity. When the bell rises 1 cm., sufficient water is automatically siphoned by a rubber tube (*K*) attached to the tank into the counterpoise weight to compensate for the apparent increase in the weight of the bell. The level of

water is maintained constant in the tank by means of a constant overflow device (*D*) which is started at the commencement of a determination. The water level in the glass limb of the counterpoise weight is used to measure the gas volume, displacement of the counterpoise being exactly equivalent to displacement of the bell. The success of this method is shown by the absence of resistance to the respiration of the animal, however much expired air has been accumulated in the bell. There is a small inertia attached to the instrument but this is a negligible factor compared with the resistance of the one-way rubber flutter valves which are employed. A maximum of 220 l. of expired air may be collected in the bell.

Procedure

The calf is confined in a pen in the lying position and a face mask made of sheet rubber is attached and sealed on with petroleum jelly. By turning the two-way cock the expired air is diverted to the spirometer, which is allowed to fill for a period of 5 min. Tap *A* (Fig. 1) is then turned to divert the expired air to the room, a weight is placed on the bell and the expired air expelled by turning tap *B*. This procedure, which rinses gas from previous determinations out of the bell and connecting pipes, is carried out three times. The water level in the counterpoise weight is then noted, tap *A* turned and expired air collected for a period of 30 min. when it is again diverted to the room. The difference in water level in the counterpoise is then read and the temperature of the gas and the barometric pressure are recorded. A sample of the air in the bell is taken and analysed for carbon dioxide and oxygen. The amount of carbon dioxide in such samples ranges from 2 to 5 % with corresponding decrements of oxygen. Calculations of the inspired air volume, the oxygen consumption and carbon-dioxide production are made according to the methods employed in human physiology. Heat production is calculated from the oxygen consumption and R.Q. using Zuntz & Schumburg's (1901) tables.

Under these conditions, where a 15 min. period is used to rinse the apparatus, the calf soon becomes adjusted to the abnormal conditions. Slight struggling often occurs during the first 5–10 min. but by that time the calf has generally made itself comfortable. One disadvantage is that in the normal resting position the calf turns its head towards its flank and lies in a curled up position. A mask attached to its face thus tends to occlude the tubes leading to the one-way valves, and so only limited head movement is allowed by placing a pillow under and on either side of the neck. A period of training does not appear necessary, since, especially during the first few days of life, calves are not highly excitable animals.

Accuracy of the procedure

The errors associated with the determination, including instrumental and analytical errors as well as any real variation in the oxygen consumption of the animal in duplicate runs, have proved small. Analysis of variance of results of duplicate determinations resulted in a standard deviation of ± 1.5 Cal./kg. body-weight/24 hr. or ± 2.9 % of the determined heat production.

SUMMARY

Apparatus and technique for the determination of the oxygen consumption and of the carbon-dioxide production of the calf during periods of 30 min. are described. The apparatus is based on that of Tissot (1904), the chief modifications being the considerable increase in its size and the inclusion of a flexible counterpoise for the bell.

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The Nutrition of the Young Ayrshire Calf

3. The Metabolism of the Calf during Starvation and Subsequent Realimentation

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 AGRICULTURAL RESEARCH COUNCIL)

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When a calf is affected by acute infantile diarrhoea, 'scouring', there is a marked fall in the amount of nutrients it absorbs from its digestive tract. This is sufficient to cause such negative nitrogen and energy balances that in many severe cases the calf is very close to a state of complete inanition. In farm practice a common and effective method of controlling this type of diarrhoea is to substitute boiled water for the normal milk allowance until the faeces become normal in appearance and then to commence realimentation very slowly. Such a method of control has the effect of substituting complete inanition for the partial inanition that results from diarrhoea. It is of some interest therefore, to study the metabolism of the calf during periods of starvation, and this paper is concerned with experiments designed to study nitrogen, sulphur, energy and mineral metabolism during short periods of starvation.

Much has been published on the effect of starvation on mature animals, especially man, but comparatively little study has been made of the effect of starvation on the metabolism of the really young animal. In the mature bovine, energy metabolism has been studied by Braman (1924), Benedict & Ritzman (1927) and Ritzman & Benedict (1938), and extensive studies of the N metabolism of the fasting steer or cow have been made by Carpenter (1927), Hutchinson & Morris (1936*a, b*) and Morris & Ray (1939). The energy metabolism of smaller mature ruminants, more especially sheep, has been studied in detail by Benedict & Ritzman (1931), Brody (1932), Ritzman, Washburn & Benedict (1936), Blaxter (1948) and Marston (1948). Little information, however, is

available on the N metabolism of the smaller ruminants during starvation (Morris & Ray, 1939). The data relating to mature ruminants have been used for comparative purposes in this study.

EXPERIMENTAL

Calves and their treatment

Two Ayrshire bull calves were each subjected to two periods of starvation. The original plan was to study starvation and recovery in each animal when at a normal weight for its age and also following a period of undernutrition when its weight would be only 50% of that which could be expected for its age. The object of such a design was twofold. Firstly, scouring in calves tends to be chronic before an acute stage is reached, and thus undernutrition for a period of time before almost complete inanition is a common phenomenon. A study of inanition following undernutrition, therefore, seemed desirable. Secondly, it has been shown by Marston (1948) that the minimal level of metabolism is reached more quickly when animals are starved following low levels of food intake than when starved following long periods of overnutrition or optimal nutrition. This may be related to the concept of a minimal base level of heat production (Forbes & Kriss, 1932; Forbes & Swift, 1941) and obviously is of considerable importance. Unfortunately one calf (no. 5) became ill during the course of the pre-starvation period for the low plane of nutrition experiment and this experiment was abandoned. The animal on slaughter was found to have greatly enlarged lymphatic glands. Metabolism results on this animal during infection are not included in this paper.

Details of the calves and of their experimental treatment are given in Table 1. The animals were confined in metabolism crates (Blaxter & Wood, 1951), the routine of feeding and collection of urine and faeces being essentially as described in that paper.

Table 1. *Details of the calves and of their experimental treatment*

Calf no.	Body-weight (kg.)	Age at commencement (days)	Experimental period (days)					
			Normal calorie intake			Low calorie intake		
			Preliminary	Starvation	Recovery	Preliminary	Starvation	Recovery
4	33.5	6	12	4	10	12	4	12
5	34.6	6	18	4	12	14	2	2

The calves were weighed every 2nd morning throughout the experiment except during the starvation periods, when they were weighed every morning.

The pulse rates of the calves were determined twice daily throughout the experiment. Additional records were taken during the starvation periods, coinciding with the respiratory exchange determinations.

Diet

The liquid diet, which was given throughout, was very similar to that described by Blaxter & Wood (1951). The main difference was an increase in its fat content from 39.2 to 42.0 g./l. and a reduction in the glucose content from 14.8 to 14.0 g./l. The amounts given to each calf are shown in Table 2.

Table 2. *Daily quantity of milk diet given to the calves during preliminary and recovery periods*

Calf no.	Normal calorie intake (kg.)	Low calorie intake (kg.)
4	3.8	3.0
5	4.0	3.2

During starvation periods, water was given instead of milk in quantities sufficient to maintain the urine volume at the pre-starvation level.

Analytical procedure

Diet. The digestibility of the dry matter, fat and total N of the diet was determined before and after starvation.

Urine. Analyses of urine were made at 2-day intervals, and daily throughout the period of fasting. Urinary sulphur was determined throughout the experiment. The urinary excretions of calcium, magnesium, sodium, potassium, chlorine and phosphorus were determined on 2-day samples of urine collected during the three periods of each experiment.

The following analytical methods were used:

Total N (Kjeldahl); urea (Van Slyke & Cullen, 1910); ammonia (Van Slyke & Cullen, 1910); creatine and creatinine (Folin, 1914); uric acid (King, 1946); allantoin (Young & Conway, 1942); purine bases (Krüger & Schmid, 1905); heat-coagulable protein (Kjeldahl); total acetone (Van Slyke, 1920); chloride (Volhard, 1878); potassium (Eden, 1943); sodium (Butler & Tuthill, 1931); calcium and magnesium (McCrudden, 1911-12); total sulphur (S. R. Benedict, 1909); inorganic sulphate, ethereal sulphate and neutral sulphur (Folin, 1905-6).

Faeces. Total N (Kjeldahl); total fat, neutral fat plus unsaponifiable residue and soaps (Saxon, 1914) were measured.

Respiratory exchange

In each of the periods of fasting, the respiratory exchange was determined on thirteen occasions, each determination being made for a 30 min. period using apparatus and technique previously described (Blaxter & Howells, 1951). On each occasion the environmental temperature was maintained as close to 20° as possible, the range being 18-21°.

RESULTS

General behaviour of the animals during fasting and when given subnormal quantities of milk

During the preliminary period, when the two calves were given quantities of diet commensurate with normal gains in body-weight, both were very lively and tended to be highly excitable, especially at feeding times. They would stand and play with their harness or their tails for quite long periods. During the first 2 days of starvation this

behaviour pattern continued, the excitability at feeding time still persisting although they were given nothing but water. Later they became more lethargic, but could hardly be called weak. When they were again placed on the normal level of feeding they recovered within a few days. During the period when the subnormal quantity of milk was given, however, activity declined markedly and the animals developed a craving for roughage, a dietary component excluded from their diets. When calf no. 4 was starved on the second occasion, chewing of the walls of the metabolism crate and of his harness increased, and on one occasion he swallowed the whole of the rubber straps supporting his faeces bag. This caused no distress, and in the subsequent recovery period he was noted to have been ruminating using the fragments of rubber tubing which he had previously ingested. At slaughter, 300 g. of chewed rubber were recovered from the rumen of this calf, and throughout the final period rubber appeared in the faeces, invalidating the determinations of carbohydrate by difference methods. Calf no. 5 also consumed small amounts of rubber tubing during the period of subnormal food intake but, although small quantities appeared in the faeces, the rumen at slaughter contained only fragments, obviously from the ends of the straps.

The behaviour of the calves on realimentation is of some interest. Realimentation of calf no. 4 was slow; he was given only half his normal quantity of diet on the 1st day following starvation, and it was noted that his urinary N remained elevated. It was thought that this might be due to the subnormal feeding, so calf no. 5 was given the whole quantity of diet immediately after the starvation period. The result was profuse diarrhoea, which lasted for several days. The same type of reaction in a mild form was shown by calf no. 4 during the second period of realimentation, when two-thirds of the normal allowance of diet was given on the day following starvation. Such alimentary disturbances after fasting are common in man (Lusk, 1928).

Body-weight

Table 3 summarizes the data for both calves. The weights of the calves on commencement of the second period of starvation were 37.95 and 41.25 kg. If the rates of gain in the preliminary period are regarded as normal, these animals should have

Table 3. *Mean daily gains or losses of weight by the calves, calculated from regression analysis of individual weights (g./day)*

Calf no.	Normal calorie intake			Low calorie intake		
	Preliminary period*	Starvation period	Recovery period*	Preliminary period*	Starvation period	Recovery period*
4	307 ± 47	-685	307 ± 42	105 ± 52	-525	130 ± 82
5	302 ± 14	-525	306 ± 26	160 ± 64	-600†	—

* Value with its standard error.

† 2 days only.

reached such weights at 27 and 31 days respectively. Their actual ages were 45 and 56 days. Thus calf no. 4 was retarded in growth by 18 days in 45, or 40 %, and calf

no. 5 by 25 days in 56, or 45 %. These retardations in growth are comparable to those observed in scouring calves. The gain in weight of the animals was not altered significantly by the interpolation of a period of starvation between two periods in which the level of feeding remained constant. There appears, therefore, to be relatively little adaptation to starvation in young animals as judged by economy in body gain following it.

In each instance, the loss of weight in starvation was severe, and there appeared to be no difference which might be judged significant between the weight losses in the two periods. The mean daily loss in weight during three experiments (second period for calf no. 5 excluded) was thus 578 ± 53 g./day.

Pulse rate

Table 4 summarizes the data. Firstly, it will be seen that the lower plane of nutrition was associated with lower pulse rates, even with calf no. 5 during the time in which he was ill. Secondly, starvation when the animal was in normal health resulted in a marked fall in pulse rate to levels in the region of 60 beats/min. The fall was naturally

Table 4. *Mean pulse rates of the calves (beats/min.)*

Calf no.	Calorie intake	8 days before fasting	First 2 days of fast	Second 2 days of fast	2-10 days following fast
4	Normal	94.6	77.5	59.7	86.7
5	Normal	89.8	73.0	64.7	88.3
4	Low	77.8	66.0	59.0	75.1
5	Low	80.9*	81.2*	—	—

* Calf abnormal.

greater when the animal had previously been maintained on a diet containing an adequate supply of energy. Lastly, almost complete recovery of the pulse rate occurred on realimentation. The detailed results plotted in Fig. 1 show the mean fall of pulse rate and return to normality for three complete periods of starvation. In this and subsequent graphs the results for calf no. 5 in the second starvation experiment have been specifically excluded. It may be concluded that during starvation pulse rate drops very markedly, reflecting the fall in the animal's metabolism. The relation between heat production and pulse rate is discussed later.

Digestibility of the diet and the excretion of faeces during starvation

The results are shown in Table 5. There was no significant change in the apparent digestibility of the diet following the first experimental starvation period. With calf no. 4 on the normal level of feeding, the digestibility of the diet increased, but the remaining results showed a decline in digestibility associated with slight diarrhoea on realimentation. These digestibility coefficients are comparable with those that may be calculated from the data of Tomme & Taranenko (1939), where the mean 'digestibility' of dietary energy was 95.1 %, but they are much lower than the earlier German results summarized by Schneider (1947), which indicate 98 % digestibility of the organic

matter and complete digestibility of the ether extractives. It is of some interest, however, that the apparent digestibility of the nitrogen of dried skim milk is of the same order as the true digestibility of the nitrogen of dried skim milk in the rat (Henry, Kon, Lea & White, 1948). Faeces continued to be excreted during the fasting period, and the amounts collected were surprisingly large. The mean daily excretion of dry matter is shown in Table 6.

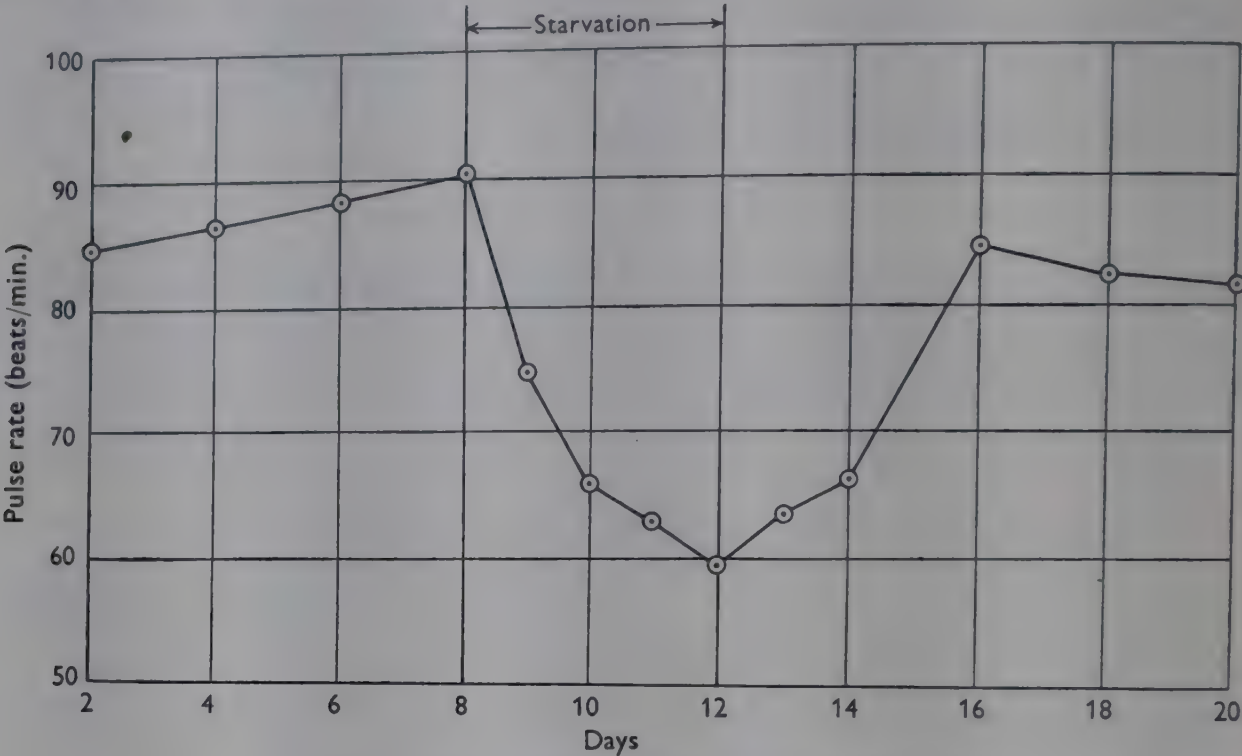


Fig. 1. Effect of starvation on pulse rate in calves.

Table 5. Mean coefficients of apparent digestibility of the diets before and after starvation

Calf no.	Level of feeding	Period	Apparent digestibility		
			Total dry matter (%)	Total fat (%)	Total N (%)
4	Normal	Preliminary	95.7	98.8	89.9
		Recovery	98.3	97.5	98.2
4	Low	Preliminary	96.7	97.1	95.1
		Recovery	94.8	93.5	93.2
5	Normal	Preliminary	95.3	93.2	90.4
		Recovery	92.3	88.7	87.4
Mean of three experiments		Preliminary	95.9	95.4	91.8
		Recovery	95.1	93.2	93.0

Table 6. Mean daily excretion of dry matter in the faeces during feeding and during starvation (g.)

Period	Calf no. 4		Calf no. 5, normal ration	Mean
	Normal ration	Low ration		
Preliminary	22.1	13.3	25.2	20.2
Starvation	8.1	13.7	10.1	10.7
Recovery	8.6	20.9	41.9	23.8

The faeces during starvation looked like normal faeces but tended to be more firm. The mean composition of the faeces during feeding and fasting is shown in Table 7.

The higher content of dry matter in starvation faeces is shown in Table 7 and it will also be noted that the composition of the dry matter of the faeces was comparable in both periods. This suggests that the starvation faeces were mainly undigested food

Table 7. *Mean dry-matter content, and mean composition expressed as percentage in dry-matter, of the faeces of the calves during feeding and during starvation*

	Normal	Starvation
Dry matter (%)	18.8	30.7
Total lipids	39.6	39.6
Soaps	25.0	23.1
Neutral fat, free fatty acids and unsaponifiable residue	14.6	16.5
Ash	20.4	19.9
Total N	5.4	5.1

residues and not metabolic products secreted into the gut during starvation. Some fraction of both the normal and starvation faeces must consist of metabolic products, but its magnitude cannot be assessed from the present results.

It may be concluded therefore that a period of starvation does not affect the digestibility of the dry matter, fat or total N of the diet subsequently determined, provided of course that the animal is not severely affected by diarrhoea.

Nitrogen balance and the biological value of the dietary nitrogen

Table 8 summarizes the N-balance results. The total urinary N of calf no. 4 increased during both periods of starvation. With calf no. 5 there was no spectacular change in N excretion in the urine on fasting. In this calf, however, the first few days of re-alimentation were associated with slight diarrhoea, and urinary N rose markedly. The total loss of N in the urine during starvation was marked. Table 9 besides summarizing

Table 8. *Nitrogen-balance results for the calves during feeding and during starvation (g./day)*

Calf no.	Level of feeding	Period	Length of period (days)	Intake	Excretion		Balance
					Faeces	Urine	
4	Normal	Preliminary	12	16.53	1.67	6.46	+8.40
		Starvation	4	Nil	0.49	9.41	-9.90
		Recovery	4	17.20	0.43	9.47	+7.30
		Recovery	6	17.76	0.25	7.85	+9.66
4	Low	Preliminary	12	13.35	0.65	7.39	+5.31
		Starvation	4	Nil	0.60	10.19	-10.79
		Recovery	4	12.42	0.60	8.88	+2.94
		Recovery	8	13.81	0.94	8.31	+4.56
5	Normal	Preliminary	14	17.27	1.65	7.33	+8.29
		Starvation	4	Nil	0.49	7.11	-7.60
		Recovery	4	15.57	2.62	10.36	+2.59
		Recovery	8	17.91	2.25	7.83	+7.83

the results on these calves, includes for comparison results obtained on other animals by different workers.

During fasting the N katabolism of the young calf is more than twice as intense as that of the cow at maturity and much greater than that of the sheep and of the goat, small ruminants of similar size. This is in substantial agreement with the endogenous N metabolism and basal energy metabolism results previously published (Blaxter & Wood, 1951) and emphasizes the intensity of metabolism in the calf.

Table 9. *The loss of body nitrogen during starvation in the young calf compared with that observed in other animals*

Animal	Loss of N (mg./kg. body- weight/day)	Reference
Young calf	259	Present work
Sheep	152	Morris & Ray (1939)
Goat	162	Morris & Ray (1939)
Pig	60	Voit (1901)
Cow	90	Hutchinson & Morris (1936a), Morris & Ray (1939)
Steer	69	Carpenter (1927)

The first 4 days of realimentation, which are shown separately in Table 8, indicate that urinary N remained high, and N retention low, in this period. Part of this in two instances was probably due to the occurrence of the slight alimentary disturbances. In the remaining instance, calf no. 4 in the first period of starvation, no diarrhoea occurred and thus such an explanation is untenable. The increase in urinary N excretion on this occasion was all at the expense of urea N and was associated with a rise in urinary ketone excretion (see below). It would appear therefore that, following a fast, excessive deamination of amino-acids for meeting energy demands takes precedence over replacement of lost tissue N. Alternatively, fasting may result in preferential demands in the subsequent period of realimentation for one particular amino-acid.

The utilization of the dietary protein was studied by calculation of the biological values of the ingested total N using the Thomas-Mitchell procedure (Thomas, 1909; Mitchell, 1923-4). Endogenous N was estimated by using the factor of 80 mg. endogenous N/kg. body-weight, and metabolic faecal N was calculated using the factor of 2.0 g. metabolic faecal N/100 g. dry matter excreted (Blaxter & Wood, 1951). The mean biological value before starvation was 72.9 and in the second period following starvation, excluding the first 4 days, it was 68.3. The difference, however, was not statistically significant (difference = 4.6 ± 1.57). The low level of food ingestion appeared to reduce the biological value of the protein.

The biological value of the proteins of dried skim milk in these experiments was thus about 70. In growing rats, the biological value of the proteins of dried skim milk is about 84 (Fairbanks & Mitchell, 1935; Sumner, 1938; Swaminathan, 1937*a, b*; Henry, Houston, Kon & Osborne, 1939; Henry *et al.* 1948). In adult man, a value of 74 has been obtained (Bricker, Mitchell & Kinsman, 1945), and in mature rats, a value of 78

(Sumner, 1938). In all these experiments the level of protein in the diet was 8 % or, with mature animals, as low as 5 %. In the present work, however, the calves were given a diet which contained 19.6 % protein on a dry basis, and 14.9 % of the total calories came from protein. When two calves were given a diet containing 25.7 % protein in the dry matter, or 19.8 % of the total calories as protein, biological values of 47.9 and 44.8 were obtained. On a diet containing 20.6 % protein in the dry matter, or 16.9 % of total calories as protein, the mean biological value was 59.7 (Blaxter & Wood, unpublished observations). These results are very comparable to those obtained by Hamilton (1938) on rats given diets containing different percentages of protein when the source of protein was dried whole egg.

Maximal biological values were thus not obtained owing to the level of protein intake having been too high, and consequently, deamination rather than storage of protein occurred. Cow's milk has a protein content of 27–30 % of the dry matter or, alternatively, protein calories constitute 23–26 % of the total calories. It would be expected that the percentage retention of N absorbed by the calf receiving its dam's milk would be much lower than could be attained on a milk with a low protein content. In this respect the conclusion of Blackwood, Morris & Wright (1936) that, relative to its N content, cow's milk is markedly deficient in calcium and phosphorus for the young calf may perhaps be modified to read that cow's milk contains an excess of N relative to Ca and P for the nutrition of the young calf, or, alternatively, cow's milk as a sole diet for the calf is grossly deficient in energy if protein retention is taken as a measure of dietary adequacy.

Distribution of nitrogen in the urine

The analytical results obtained on each calf are summarized in Table 10. Fig. 2 shows the mean changes in distribution in more detail and is referred to later.

Table 10 and the average results in Fig. 2 show that urea excretion increased during starvation. This was marked with calf no. 4 on both occasions but was negligible with calf no. 5, a result in agreement with the N-balance results previously discussed. Fig. 2

Table 10. *Mean daily excretion of nitrogen in different nitrogenous metabolites in the urine of the calves during feeding and during starvation (g.)*

Metabolite	Calf no. 4 Level of feeding						Calf no. 5 Normal level of feeding		
	Normal			Low			Normal level of feeding		
	Preliminary period (8 days)	Starvation period (4 days)	Recovery period (10 days)	Preliminary period (6 days)	Starvation period (4 days)	Recovery period (10 days)	Preliminary period (10 days)	Starvation period (4 days)	Recovery period (8 days)
Urea	4.62	7.34	6.11	4.68	7.10	4.74	4.80	5.00	3.69
Ammonia	0.39	0.90	0.89	1.01	0.58	1.18	0.66	0.55	1.27
Total urea and ammonia	5.01	8.25	7.00	5.69	7.69	5.92	5.46	5.55	4.96
Creatinine	0.365	0.379	0.285	0.232	0.236	0.229	0.467	0.379	0.441
Creatine	0.124	0.214	0.098	0.125	0.197	0.232	0.108	0.377	0.252
Uric acid	0.038	0.057	0.031	0.026	0.048	0.029	0.037	0.048	0.042
Purine base	0.131	0.148	0.193	0.216	0.206	0.252	0.146	0.138	0.211
Allantoin	0.606	0.520	0.373	0.476	0.569	0.583	0.605	0.507	0.645
Total purine	0.775	0.725	0.597	0.718	0.823	0.864	0.788	0.693	0.898
Residual N	0.729	0.117	0.233	1.039	1.227	2.101	0.483	0.186	1.857

shows that urea excretion fell slowly following the fast, again in agreement with the results for total N metabolism. Ammonia excretion was not markedly affected by starvation. This differs from the effect of starvation in man (Cathcart, 1907) where an increase in ammonia excretion counteracts the marked acidosis.

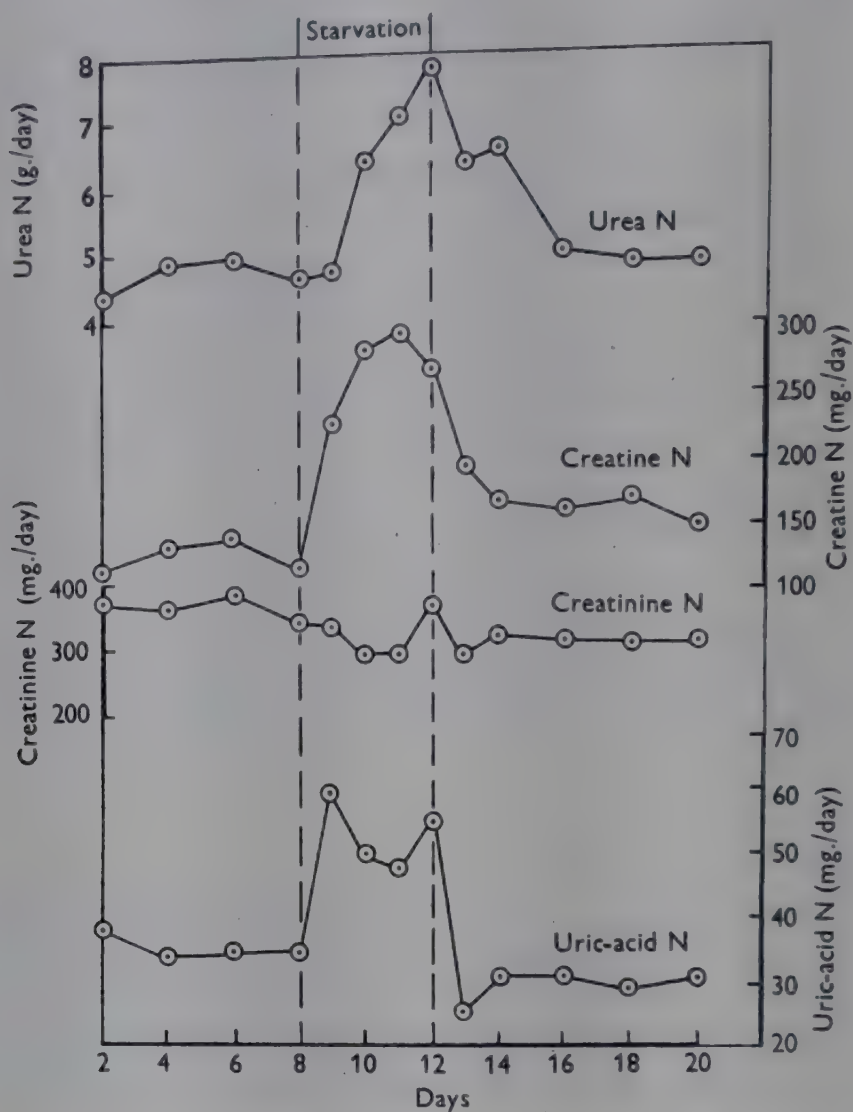


Fig. 2. Effect of starvation on the urinary excretion by calves of urea, creatine, creatinine and uric acid.

The excretion of creatinine declined very slightly throughout, as shown in Fig. 2, there being a slight fall in the recovery period of calf no. 4 in the first starvation experiment, a change of doubtful significance. This is in agreement with Folin's (1905) contention of a constant endogenous metabolism and constancy of creatinine elimination. Creatine excretion rose when the calves were starved. This was true even of calf no. 5, which showed no pronounced change in total N excretion during fasting. The purine bases showed no constant change during starvation, allantoin N did not change, and uric-acid excretion increased, during the starvation period in both animals although the amount of N involved was small. The total purine-N excretion was not affected by starvation, the small variation being commensurate with the day-to-day variation in urinary metabolite excretion met in such studies. The uric-acid N excretion is also shown in Fig. 2.

The residual N, which represents largely amino-N, with the small amounts of N present as protein (albumin) in amines and other compounds, was variable. This was undoubtedly partly due to analytical errors, since the determination of this fraction involves eight separate determinations of N or N-containing compounds. Nevertheless, it was clear in the second experiment with calf no. 4, and more especially in the recovery period of this experiment, as well as in the recovery period of the first experiment with calf no. 5, that the residual N was unduly large. Part of this was traced to an excretion of a heat-coagulable protein. Analytical data were not obtained throughout, but analyses of the urine of calf no. 5 during the preliminary period on the low-calorie diet indicated that up to 1.2 g. N, equivalent to 7.5 g. protein, were being lost daily by the kidneys in the form of a heat-coagulable protein. In any case, there was no indication that any major fraction of the urinary N of starvation was present in the undetermined moiety.

Table II compares the N distribution during starvation of the urine of the calf with that of the adult ruminant.

Table II. *Distribution of nitrogen in different nitrogenous metabolites in the urine of starving calves, goats, sheep and cows expressed as percentages of total urinary nitrogen*

Metabolite	Calf	Goat*	Sheep*	Cow*
Urea	72.49	64.42	57.56	30.39
Ammonia	7.57	3.64	3.87	31.77
Total urea and ammonia	80.06	68.06	61.43	62.16
Creatinine	3.70	6.12	7.88	7.35
Creatine	3.06	3.63	4.86	4.77
Uric acid	0.57	1.08	1.44	0.58
Purine base	1.83	6.16	4.66	2.14
Allantoin	5.95	11.12	18.41	14.24
Total purine	8.35	18.48	24.51	16.96

* Results of Morris & Ray (1939) recalculated and expressed in a form suitable for comparing with present results for calves.

The table shows that the starvation metabolism of the calf differs from that of the cow mainly in that the percentage of creatinine N and total purine N of the total N is only half that found in the same species at maturity or in mature ruminants of similar body-weight. The urea and ammonia N constitutes a considerably greater percentage of the total N in the calf than in mature ruminants. Before discussing this further it is of interest to compare the endogenous N excretion and the N excretion during starvation of the calf, as shown in Table 12. The endogenous N metabolism results in Table 12 are those of Blaxter & Wood (1951); the protein metabolism results are those determined in the preliminary periods of the three present experiments. It is clear that the starvation metabolism must have included the endogenous N metabolism and that the difference between starvation metabolism and endogenous metabolism represents the breakdown of body tissues to provide energy for vital processes during energy deprivation. Of the total increase in N excretion above the endogenous level, 91.1 % was thus due to the excretion of urea and ammonia and 2.8 % to the excretion of creatine. It is probable that these values may be too low, since it was found that total energy metabolism declined during fasting (see below) and thus the endogenous metabolism of the

Table 12. *Nitrogen metabolism of the calf during starvation, its endogenous metabolism and urinary nitrogen excretion during protein ingestion*

Metabolite	Endogenous N excretion* (mg./kg. body-weight/day)	N excretion during starvation (mg./kg. body-weight/day)	Urinary N excretion during protein feeding (mg./kg. body-weight/day)
Total N	81.9	245.6	203.0
Urea	33.2	178.1	129.3
Ammonia	14.5	18.6	19.1
Total urea and ammonia	47.7	196.8	148.4
Creatinine	10.1	9.1	9.8
Creatine	2.9	7.5	3.2
Uric acid	1.3	1.4	0.9

* Results of Blaxter & Wood (1951) recalculated.

calves may also have declined. The slightly lower creatinine excretion per kg. body-weight during fasting adds weight to this suggestion.

This large increase in N excretion during fasting appears compatible with an hypothesis that tissues comparable in composition to muscle substance are katabolized. Table 13 compares the composition of muscle with that of the excess N in the urine, and it will be noted that the katabolism of 0.48 g. muscle/kg. body-weight would yield the same quantity of total N and creatine N and slightly less N as urea and ammonia than was found in starvation.

Table 13. *The composition of muscle (Lusk, 1928) and the increase in the nitrogen excretion during starvation over the endogenous level in the calf*

Constituent	Amount present in muscle (%)	Amount in 0.48 g. muscle* (mg.)	Excess urinary N (mg./kg. body-weight)
Protein N	3.2	153.9	149.1†
Creatine N	0.096	4.6	4.6
Total N	3.4	163.7	163.7

* See text above.

† Sum of urea and ammonia N (amino-N not estimated).

It may be noted from Table 12 that the katabolism of exogenous protein within the body was associated with a small elevation of creatine elimination (1 %) but mainly with an increase in urea and ammonia elimination. It is possible that this small increase in creatine excretion may be due to the dried skim milk in the diet having contained creatine N amounting to 1 % of the total N (Bleyer, 1930).

The differences between the N distributions in the urine of the animals as shown in Table 11, would appear therefore to be entirely due to a proportionately greater katabolism of body protein in the young calf, thus 'diluting' the endogenous moieties of the total N excretion.

The diet used for the experimental calves was virtually purine-free, and yet starvation

did not increase purine excretion, save for a small increase in uric-acid elimination. The same conclusion may be drawn from the calculation that the increased excretion of N above the endogenous level during starvation can be accounted for to the extent of 94 %, leaving only 6 % to be accounted for in terms of purine and residual N. This suggests that in the calf during fasting there is no extensive katabolism of nucleoprotein materials and that the cells of the body remain intact. Morris & Ray (1939) and Hutchinson & Morris (1939*b*) have concluded that in the ruminant there is a marked reduction of nuclear cell metabolism during fasting. These conclusions were based on the reduction of purine-N excretion in the urine when ruminants were starved after having received diets containing normal foods and hay. Such diets are not purine-free and the reduction Morris and his colleagues noted merely reflects a decrease in the exogenous excretion of nucleic-acid derivatives. It is of some interest to compare the distributions of purine N in the urine of the calves and of mature cows. This comparison is shown in Table 14. It will be noted that the calf excretes a slightly higher percentage of its total purine as purine bases than the mature animal. It is also evident, as previously emphasized, that while the mean daily excretion of purine N remains nearly constant when the calf is starved the proportion present as uric acid increases.

Table 14. *Mean percentage distribution of the purine nitrogen in the urine of the calf, cow and goat before and during starvation*

Constituent	Calf		Cow*		Goat*	
	Before starvation	During starvation	Before starvation	During starvation	Before starvation	During starvation
Purine base	22	22	12	15	14	29
Uric acid	4	7	5	3	6	6
Allantoin	74	71	83	82	80	65
Mean daily excretion of purine N (mg.)	760	747	—	—	—	—

* Morris & Ray (1939).

Excretion of sulphur in the urine and the partition of the urinary sulphur

The mean results are shown in Table 15. It is clear from this table that an increase in total S excretion occurred during starvation, smaller in calf no. 5 than in calf no. 4, a result in agreement with the N-metabolism results. The partition of the urinary S

Table 15. *Total urinary excretion of sulphur by the calves and the nitrogen:sulphur ratios during feeding and during starvation*

Period	Calf no. 4 Level of feeding		Calf no. 5 Normal level of feeding	Mean
	Normal	Low		
	Total excretion of S (mg./day)			
Preliminary	323	413	326	370
Starvation	611	607	403	541
Recovery	476	394	229	366
	N:S ratio			
Preliminary	22.7	19.0	22.0	—
Starvation	16.6	16.7	17.7	—

recorded in Table 16 shows that the major part of the increase in excretion occurred in the inorganic fraction, and that the neutral fraction and the ethereal-sulphate fraction remained constant.

Table 16. *Mean values for the partition of the urinary sulphur of calves during feeding and during starvation*

Fraction of sulphur	Preliminary period (mg./day)	Starvation period (mg./day)	Increase (mg./day)
c Inorganic	210.4	382.2	+171.8
Ethereal	66.3	63.3	-3.0
Neutral	93.8	95.1	+1.3
Total	370.5	540.6	+170.1

The constancy of the neutral S fraction is in agreement with the contention that it is an endogenous fraction, though the experiments of Amann (1933) suggest that its excretion is by no means constant, increasing markedly when very high protein diets are given. The constancy of the ethereal-sulphate fraction was not expected. It was thought that there would be a reduction in the quantity of phenols formed by putrefaction and stasis in the gut on starvation with a consequent decline in the sulphate-ester excretion. Possibly the continuing excretion of faeces during fasting indicates that there are present in the intestinal tract sufficient food residues to give rise to phenols in comparatively large quantities.

The ratio N:S in the urine of man during starvation varies between 15 and 17 (Lusk, 1928; Benedict, 1915; Cathcart & Green, 1913). The N:S ratio in skeletal muscle is 13.4 (Wilson, 1925). In the calf the N:S ratios are comparable to those found in starvation metabolism in man but are higher than would be expected if muscle proteins and their constituent S-containing amino-acids were the source of both the N and the S. The explanation probably is that neither the total S nor the total N entirely originates in muscle katabolism (see Table 12 and subsequent discussion), a small part in each instance coming from the so-called endogenous metabolism. If it is assumed that the excretion of neutral S is a measure of a minimal S excretion, and the excretion of N above the endogenous level is taken as representing katabolism of body protein, the ratio of non-endogenous N to non-endogenous S is 13.8, a value in fair agreement with Wilson's value for the N:S ratio in muscle.

It may be concluded, therefore, that there is an increase in S excretion during fasting following a milk-protein diet, the increase being entirely in the inorganic fraction, and that, subject to one assumption, the excretion of S is compatible with the hypothesis that body protein is being katabolized in large amounts.

Excretion of acetone in the urine

The daily excretion of acetone is shown in Fig. 3, and the mean results are summarized in Table 17. It is clear from Fig. 3 and Table 17 that no ketosis occurred on starving the young calf. This agrees with the results obtained in the mature ruminant by Sjollem & van der Zande (1923), Carpenter (1927) and Hutchinson & Morris (1936*b*),

none of whom observed any ketosis in cattle on starvation. This result in the young calf, which is to all intents a non-ruminant, simple stomached animal, is of considerable interest, for in man many g. of ketone bodies are excreted during fasting. The extent of the ketosis is much smaller in the poorly nourished individual (Deuel & Gulick, 1932), suggesting that in the calf, an animal possessing only small fat reserves, ketosis would not be a symptom of starvation. In this respect, however, infants and children evidently develop a more intense ketosis on starvation than adults (Gamble, Ross & Tisdall, 1923).

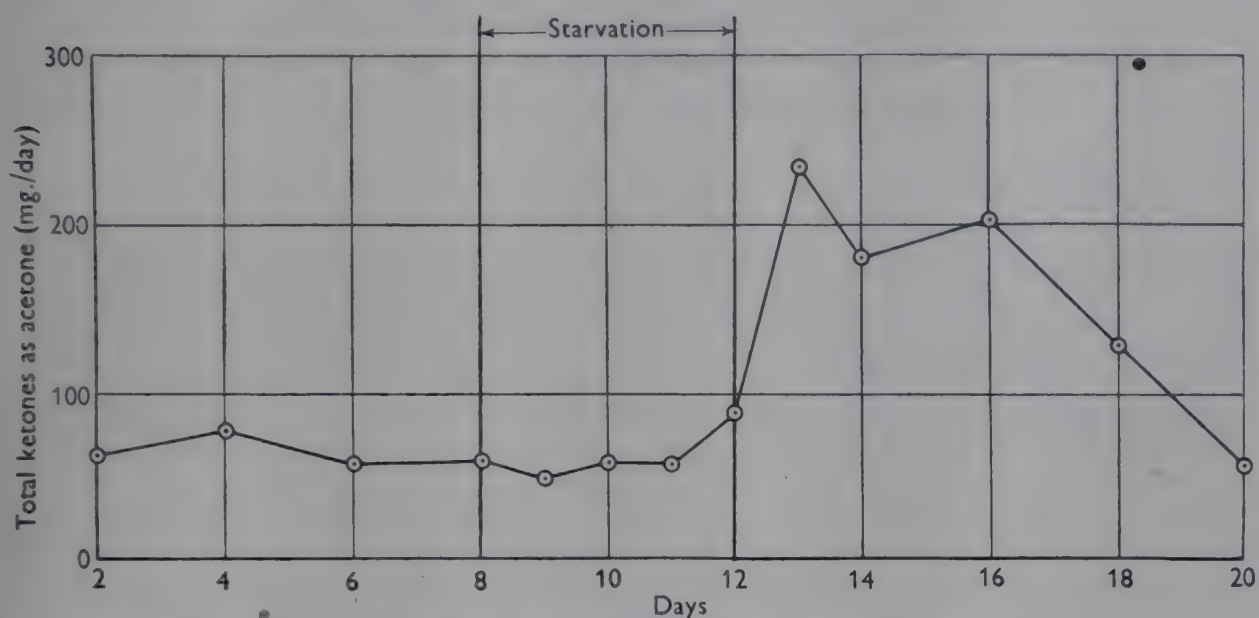


Fig. 3. Effect of starvation on the urinary excretion by calves of ketone bodies.

Table 17. *Daily excretion of ketone bodies in the urine of the calves during feeding and during starvation, expressed as acetone in mg./day*

Period	Calf no. 4 Level of feeding		Calf no. 5 Normal level of feeding	Mean
	Normal	Low		
Preliminary	60.7	58.5	68.7	62.6
Starvation	49.4	54.3	89.2	64.3
Recovery	95.7	134.2	120.3	116.7

It is of interest that an increase in urinary acetone excretion occurred on realimentation. This suggests that there is a slight disturbance of carbohydrate metabolism at this time. It may be suggested that the large demand for glucose to replenish the depleted glycogen resources of the animal results in an increase in fatty-acid oxidation following fasting.

Excretion of minerals in the urine

As already stated, calcium, magnesium, sodium, potassium, chlorine and phosphorus were determined on 2-day samples of urine collected during the three periods of each experiment. The results are shown in Table 18. Balances were not determined, and it must be remembered that the sampling errors involved in obtaining these results may have been high.

Table 18. *Daily excretion of mineral elements in the urine of the calves during feeding and during starvation (mg.)*

Calf no.	Level of feeding	Period	Cl	P	Na	K	Mg	Ca
4	Normal	Preliminary	2467	227	914	2678	62	149
		Starvation	664	312	661	1493	34	75
		Recovery	2884	399	1398	2780	30	106
4	Low	Preliminary	2003	245	529	2027	13	135
		Starvation	274	143	330	1569	30	33
		Recovery	2614	345	876	3291	18	87
5	Normal	Preliminary	3057	182	338	3145	45	94
		Starvation	140	184	—	1315	8	22
		Recovery	2379	28	313	1496	18	71
Mean loss/day during starvation			326	213	495	1456	24	43

It is clear that there was a reduction in the excretion of Cl, K, Na and Ca during starvation and no constant change in the other elements. The mean daily loss during starvation may be used to indicate the extent of the katabolism. If only muscle tissue was being broken down, and if the K loss is assumed to have come entirely from muscle, one can estimate the approximate excretion of other elements which could have come from the muscle substance. This can only be an approximation, for the complete analytical figures for muscle are very few and were mostly obtained many years ago, whereas the urinary excretion as determined is subject to a fairly large sampling error. By use of ratios, however, part of the sampling error is minimized. The final results of the calculation are shown in Table 19.

Table 19. *Calculation of approximate amounts of body protein katabolized, based on the analysis of muscular tissue and on urinary excretion of the calves*

Element	Amount in muscle when 1500 mg. K are present* (mg.)	Mean daily excretion in the urine (mg.)	Conclusion
K	1500	1456	—
Na	400	495	Loss of extracellular fluid
Cl	350	326	—
Ca	35	43	No loss of minerals from bone
Mg	105	24	Retention of essential enzyme systems of the cell
P	750	213	

* See text above.

It would appear from Table 19 that there was no katabolism of bone minerals during starvation. This is not in agreement with some of the data obtained on human beings (Peters & Van Slyke, 1931) but, as has been pointed out, part of the bone loss in man may be due to the accompanying acidosis. Hawk, Oser & Summerson (1947), however, point out that long periods of starvation in the dog (up to 104 days) do not cause any marked loss of minerals from the bone.

The larger quantity of Na present in the urine suggests a loss of extracellular fluid

during starvation. The smaller quantities of Mg and P present in starvation suggest that there was no extensive loss of nucleoprotein material and that the enzyme systems of the cell remain intact. This is in substantial agreement with the results previously reported on the absence of an increase in purine katabolism during a fast.

Energy metabolism and respiratory exchange

Accuracy of the determinations. In that the interpretation of the results of the respiratory-exchange determinations depends largely on the accuracy with which the determinations were made it is essential to have information on the variation associated with each determination. It may be stated at the outset that the method

Table 20. Regression equations relating functions measured during determinations of starvation respiratory exchange to length of starvation, with coefficients of variation estimated by analysis of variance of the regression

Function	Equation	Percentage decline/ day	Variance ratio (e^{2z})	Coefficient of variation (%)
Carbon-dioxide production (l./hr.)	$x = 10.117 - 0.600D$	5.93	736.4	1.4
Oxygen consumption (l./hr.)	$x = 12.997 - 0.755D$	5.81	233.2	2.0
R.Q.	$x = 0.784 - 0.006D$	7.96	3.7	1.8
Heat production (Cal./hr.)	$x = 61.67 - 3.76D$	6.10	502.4	1.4
Pulse rate (beats/min.)	$x = 79.7 - 6.38D$	8.00	498.3	2.0
Respiratory rate (respirations/min.)	$x = 16.6 - 1.15D$	6.93	7.0	13.9
Minute volume of respiration (l.)	$x = 4.40 - 0.032D$	7.36	17.8	13.8
Body-weight (kg.)	$x = 35.88 - 0.655D$	1.83	14.9	1.6

D: days of starvation.

adopted for determination of respiratory exchange was extremely sensitive. Slight head movements of the calf in a duplicate run invariably could be detected in a higher consumption of oxygen and production of carbon dioxide. The accuracy of the method was determined by analysis of variance, computing the coefficient of variation from the mean and the standard deviation of the residuals from a fitted linear regression. The results for calf no. 4, which are typical, are shown in Table 20. From the results in that table it is clear that the errors attached to the determinations were very small. For oxygen consumption, heat production and carbon-dioxide production the errors expressed as a percentage of the mean are all less than 2. The respiratory rate and ventilation rate per min. were slightly more variable, but even so were well within the range of variability one might expect in a function under partial voluntary control.

The course of heat production during fasting with reference to the constancy of the basal metabolism of the calf. From Table 20 it is clear that the linear component of the regression was very highly significant in calf no. 4 and that the residual variance was very small. In both calves heat production following feeding declined slowly over the whole 4 days of observation. The same was true of each function studied, pulse rate, respiratory rate and minute volume. The data relating to each animal are shown in Table 21.

From the equations in Table 21 it can be seen that both calves showed a marked

decline in heat production throughout the fasting period. There was no indication at any time that a constant level of metabolism had been reached, and the regression equations were not in any respect non-linear, as can in fact be inferred from the errors shown in Table 20. In every instance the decreases in oxygen consumption, heat production and body-weight with continued fasting were all highly significant, P being always smaller than 0.01 and sometimes smaller than 0.001.

Table 21. *Regression equations showing the fall in heat production of the calves with continued starvation, the fall in oxygen consumption and the decline in body-weight*

Calf no.	Level of feeding	Function	Regression equation	Daily percentage decline
4	Normal	Oxygen consumption (l./hr.)	$x = 13.00 - 0.600D$	5.81
		Heat production (Cal./hr.)	$x = 61.67 - 3.76D$	6.10
		Body-weight (kg.)	$x = 35.88 - 0.655D$	1.83
4	Low	Oxygen consumption (l./hr.)	$x = 13.28 - 0.454D$	3.42
		Heat production (Cal./hr.)	$x = 53.67 - 3.32D$	6.19
		Body-weight (kg.)	$x = 38.83 - 0.525D$	1.35
5	Normal	Oxygen consumption (l./hr.)	$x = 14.86 - 0.68D$	4.58
		Heat production (Cal./hr.)	$x = 64.58 - 4.36D$	6.76
		Body-weight (kg.)	$x = 38.89 - 0.315D$	0.81

D : days of starvation.

The heat production was computed using the mean excretion of N/day. The rate of decline in heat production was much greater than the decline in body-weight, which means that, not only the metabolism of the animal per kg. body-weight declines markedly during fasting, but that its metabolism per unit of surface area declines at an even greater rate, in that surface area tends to be proportional to a fractional power of body-weight between 0.6 and 0.8. It will be noted that the rate of decline of heat production was the same whether or not calf no. 4 had received an adequate or a reduced amount of diet, and that the rate of fall in the heat production of calf no. 5 was also similar.

It is clear that this fall in heat production of calves during starvation is not only highly significant in the single individual but is reproducible as between individuals and not affected by the earlier nutrition of the individual within the limits employed. These results are in marked contradistinction to those found with man and mature animals. In the first place, though in man there is a fall in total heat production in long-continued fasting, fasting for 1 week does not result in a large fall in metabolism per kg. body-weight (Lusk, 1928; Benedict, 1907, 1915; Lehmann & Zuntz, 1893). In the cow, the metabolism/kg. body-weight falls during the 1st day of starvation, largely owing to the long period needed to reach a postabsorptive state. The metabolism of the animal does not appear to decline markedly once this level has been attained (Benedict & Ritzman, 1927). In sheep, the observations of Blaxter (1948) and Marston (1948) indicate that a plateau in metabolism occurs. Prolonged starvation in the rat, however, reduces heat production whether expressed per kg. body-weight or per sq.m. body surface (Benedict & Fox, 1934), and in mice a marked fall in metabolism, as judged by body-temperature changes, occurs during continued fasting (Buschke & Vasarhelyi, 1932).

Data relating to this decline in heat production in other species are given in Table 22. Statistical analyses were made of the published results by the methods used in dealing with the calf results.

Table 22. *Regression of heat production (Cal./kg. body-weight) on length of fast in days; a comparison of the calf with man and the steer*

Species	Reference	Regression equation	Length of observation (days)	Statistical significance
Calf	Present results	$\text{Cal.} = 37.03 - 1.98D$	4	Very highly significant
Man	Benedict (1907)	$\text{Cal.} = 32.28 - 0.66D$	5	N.S.
Man	Lusk (1928)	$\text{Cal.} = 30.97 - 0.39D$	6	N.S.
Steer { C D E	Benedict & Ritzman (1927)	$\text{Cal.} = 16.62 - 0.47D$	6	N.S.
		$\text{Cal.} = 17.12 - 0.58D$	6	N.S.
		$\text{Cal.} = 22.4 - 0.04D$	4	N.S.

D: days of starvation. N.S.: not significant.

The second aspect of this decline in heat production following fasting is that it is not affected to any appreciable extent by the earlier nutritional level of the individual. There is a reduction of the intercept when $D=0$ (Table 22), but no change in the percentage decline. Marston (1948) has shown that heat production of sheep fed on a high plane of nutrition fell to a constant basal level more slowly (over a period of 7 days) than the heat production of animals fed on a submaintenance diet which reached constancy after only 2 days of fasting. Marston has attributed this difference in the shape of the curve of heat production during fasting to the failure of the animals given the higher level of energy to reach a postabsorptive state.

The reason for this decline in metabolism in prolonged fasting in the calf is not clear. The most logical supposition would be that a postabsorptive state had not been reached in the calf starved for 4 days. This is not supported by the results for the following reasons: (1) The respiratory quotient did not show any marked decline during the fast. This was also true of the non-protein R.Q. (2) The peak of creatine excretion in the urine occurred early in the starvation period, indicating that the animal was very close to a postabsorptive state even on the 1st day of starvation. (3) There was no significant difference in the rate of decline in metabolism following different levels of food intake, as has been shown to occur in the sheep. (4) The fall in metabolism was not exponential and the experimental points did not deviate from a linear rate of decline. If the decline in metabolism was due to the metabolism of food residues an asymptote would be expected.

The decline in metabolism was thus not due to failure to reach the postabsorptive state. This is reasonable in so far as the rat reaches a basal level of metabolism at about 14 hr. (Wesson, 1930-1) and man at about 12 hr. It is only herbivora that take 72 hr., or more.

A likely explanation is that there is a marked reduction in muscular tone and in the small, almost involuntary, skin and muscle movements during prolonged fasting in the calf. Body temperatures were unfortunately only recorded on one occasion, when, at

the end of fasting, a value of 99.4°F. was obtained, compared with a previous value of 101.5°F. at the low level of feeding.

Relation between the heat production of the calf and its cardio-respiratory activity. As heat production fell, the pulse rate, respiratory rate and minute volume of the respiration also fell in both the calves. That the pulse rates of sheep and cattle are approximately proportional to their metabolism is well known and has recently been studied by Blaxter (1948). Fig. 4 shows the relationship in calf no. 4, with the results pooled for both periods of starvation. The equation relating metabolism to pulse rate was:

$$\text{Cal./hr.} = 10.46 + 0.644 \times \text{pulse rate.}$$

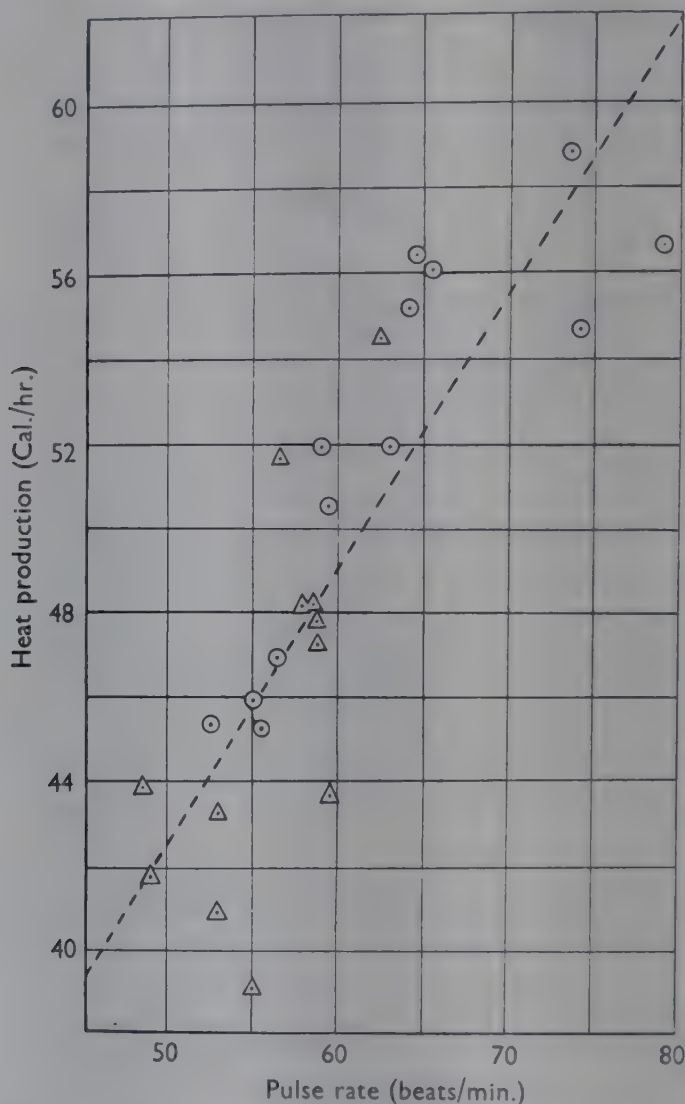


Fig. 4. Relation between pulse rate and heat production in calf no. 4. $\circ$, first starvation experiment; $\triangle$, second starvation experiment.

This regression was very highly significant statistically. By calculating this equation to the basis of Cal./sq.m. body surface/24 hr. it may be compared with similar equations computed for the sheep and the steer. The transformed equation is shown in Table 23.

It would appear that the relation between pulse rate and metabolism is fairly similar in the young calf and the adult sheep, animals of about the same size, but that in the steer the heat production is proportionately much greater per unit of body surface for comparable pulse rates.

Table 23. *Relation between heat production and pulse rate in the calf, the sheep and the steer*

Species	Weight (kg.)	Equation	Heat production (Cal./sq.m. body surface/24 hr.), with pulse rate beats/min.		
			50	70	90
Calf*	40	$M = 13.6P + 220.0$	900	1172	1444
Sheep	40	$M = 17.2P - 117.5$	743	1086	1431
Steer	400	$M = 16.4P + 831.4$	1650	1978	2306

* Surface area taken to be 1.14 sq.m.

P: pulse rate (beats/min.). M: heat production (Cal./sq.m. body surface/24 hr.).

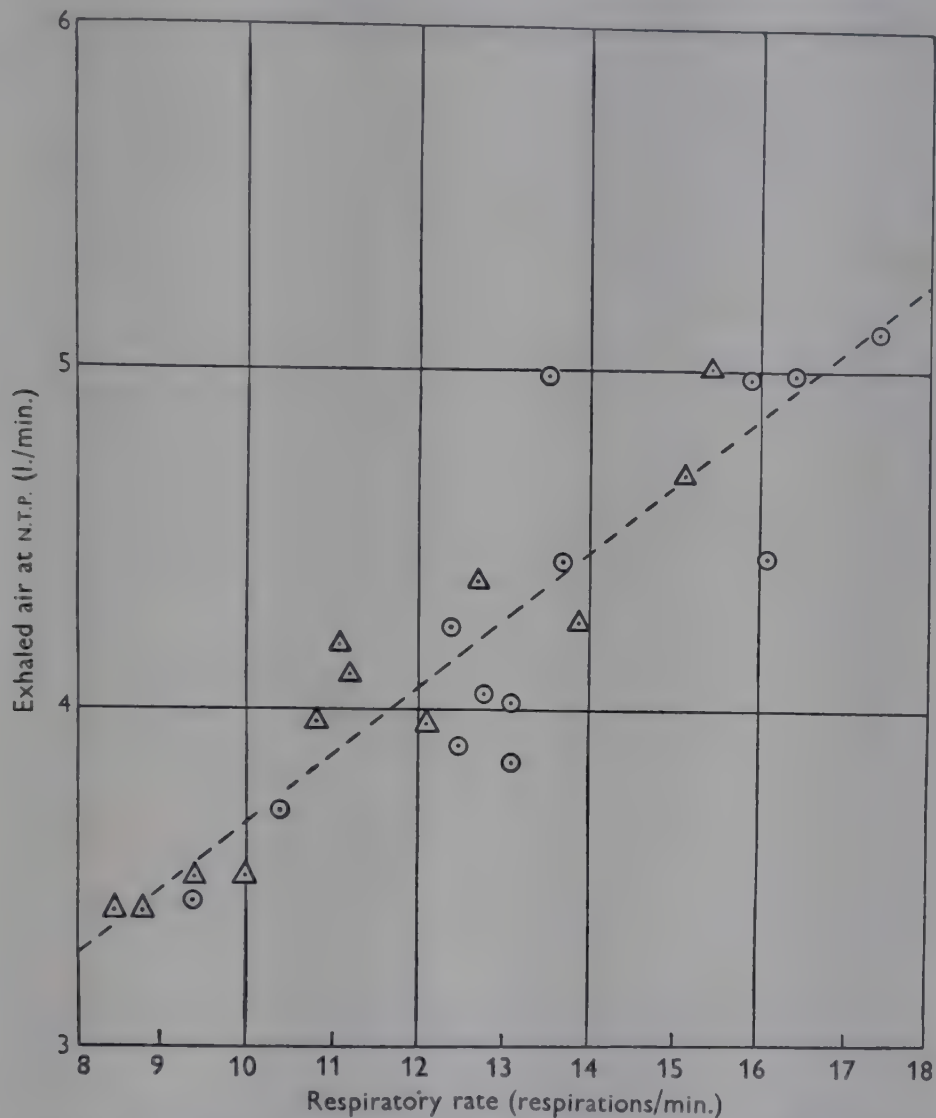


Fig. 5. Relation between respiratory rate/min. and the minute volume of the exhaled air in calf no. 4. ○, first starvation experiment; △, second starvation experiment.

The relation of the minute volume to the respiratory rate of the calf during fasting is shown in Fig. 5. From this it is clear that an increase in respiratory frequency is associated with an increase in minute volume. This is not a direct proportionality, for the tidal air of the respiration declines with increasing respiratory rate as shown in Fig. 6. The equation for the straight line regression is:

$$\text{Tidal air, in ml. at N.T.P.} = 482.4 - 11.75 \times \text{respiration rate.}$$

This equation applies only within the limits of 7–20 respirations/min. and within this

range may be regarded as highly significant statistically. In the sheep, an animal of closely similar size, the equation relating tidal air to respiratory frequency has a regression coefficient of only 1.9, compared with 11.8 in the calf. The sheep experiments were all associated with respiratory rates greater than 25/min., even during prolonged fasting, and the two sets of observations are probably not fully comparable (Blaxter, 1948).

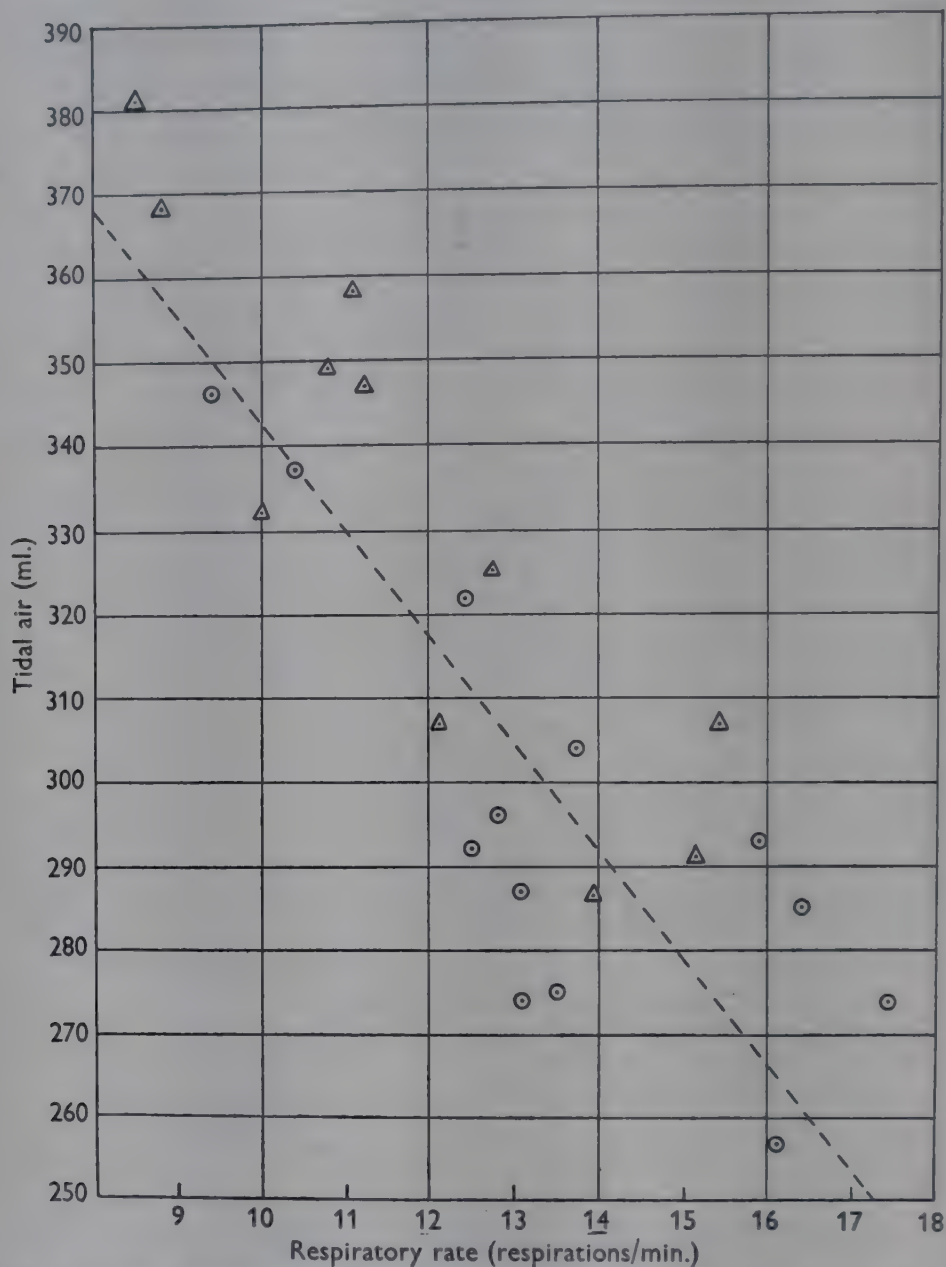


Fig. 6. Relation between respiratory rate/min. and tidal air in calf no. 4. $\odot$, first starvation experiment; $\triangle$, second starvation experiment.

DISCUSSION

Materials katabolized during fasting in the calf, and the loss in body-weight

The respiratory quotient of an animal when corrected for total urinary N excretion using the factors of Loewy (1911) or of Kriss & Miller (1934) may be used to compute the proportions of fat and carbohydrate oxidized in the body. This method has recently fallen into disrepute (Soskin & Levine, 1946) largely owing to the realization that reactions other than simple total oxidation of fat and carbohydrate are always occurring. As pointed out by Dewar & Newton (1948-9), over long periods of time

this criticism is not valid, for the intermediate side-reactions resulting in abnormal respiratory quotient will be cancelled out. In long-term experiments carried out over 24 hr. periods, the R.Q. gives substantially the proportion of energy derived from the ultimate oxidation of fat and carbohydrate. In the present experiments the experimental periods were only 45 min. in total duration (including preliminary periods) and 'abnormal' R.Q.'s might therefore be expected. In the first experiment with calf. no. 4, the non-protein R.Q. remained high throughout, indicating that in the early stages of the fast 10-12 % of the heat production was arising from complete oxidation of carbohydrate. In the remaining experiments non-protein R.Q.'s were quite normal, varying within small limits. On occasions, however, values below 0.707 were obtained, indicating either that complete collection of the carbon dioxide produced was not obtained owing to underventilation or that momentarily the determination had coincided with a phase of intermediary metabolism characterized by a low apparent non-protein R.Q. In no instance was the extremely wide variation of R.Q. met by Werthessen (1937) or Markowitz (1946) ever observed, any variation from the general trend of the non-protein R.Q. with time being within limits of ± 0.05 .

It would appear therefore that the present results may be used to compute the fat and carbohydrate oxidized or dissimilated, and for the reason given above, the results for calf no. 4, period 1, have been excluded as far as a partition of the non-protein heat production is concerned. Table 24 summarizes the results of the calculation of the

Table 24. *Estimate of constituents lost daily from the body of the calf during starvation; calf no. 4, period 2 and calf no. 5, period 1*

Constituent of body	Daily loss (g.)
Body-weight	525
Body fat	99
Body protein	54
Body carbohydrate	13
Extracellular water	39
Intracellular water	326
Total weight loss accounted for	531

materials katabolized. The extracellular and the intracellular water losses are based on the molal quantities of sodium and potassium excreted in the urine during fasting and calculated using the equations of Gamble *et al.* (1923). The table shows that by far the greatest loss to the body is water and that most of this is from the cells rather than from the intercellular spaces. The loss of extracellular water is small and indicates only a slight dehydration of the animals. The loss of body fat of nearly 100 g./day is the mean loss on the 2nd day of starvation. Towards the end of the starvation period, on the 4th day, proportionately far more protein (up to 24 % of the total calories in calf no. 4) was lost to the body. In this respect, it has to be remembered that not the whole of the urinary N is necessarily a reflexion of protein katabolism. This has been adequately discussed in the section dealing with urine composition (p. 39), and serves to indicate that the protein value in Table 24 is probably too high an estimate, part of the loss of N being of body non-protein nitrogen constituents.

We have not determined the source of the katabolized protein, fat and carbohydrate, but have shown that there is no important loss of nucleic-acid derivatives on fasting the calf. Kosterlitz & Campbell (1945) have recently reviewed aspects of the loss of N-containing compounds from the body in starvation and have pointed out the relatively large loss of N from the liver of the fasting animal. This loss is accompanied by a loss of ribonucleic acid from the liver cytoplasm (Kosterlitz, 1944; Davidson & Waymouth, 1944*a, b*). There is no reason to believe, on the basis of chemical analysis, that in the loss of muscle substance during starvation nucleic acid and protein are not lost simultaneously. On the other hand, in muscle in starvation there is both an absolute and a relative increase in the number of cell nuclei (Roche & Hoerner, 1933), and in the liver only the labile liver cytoplasm is reduced (Kosterlitz, 1944; Campbell & Kosterlitz, 1947) while the number of cell nuclei with their large store of deoxyribonucleic acid remains intact (Davidson & Waymouth, 1944*a*; Davidson, 1945).

In the calf there was no indication of an acceleration of purine excretion during starvation, indicating that adenine and guanine were not arising from cytoplasmic ribonucleic acid or nuclear deoxyribonucleic acid in large quantities. This is compatible with the contention that the major portion of the N loss was from the non-nuclear portion of the muscles, with relatively very much smaller portions arising from the liver.

Fasting the calf as a method for the control of diarrhoea

From the above discussion it is clear that fasting the young calf causes marked losses of its body protein and fat, and that, because its metabolism is more intense than that of the older animal, its losses are proportionately more severe (see Tables 9, 11 and 22). A higher percentage of the total heat loss of the calf comes from the degradation of body protein than in the mature animal. This is in complete accord with the classical work of Voit (1901) who showed that the quantity of the protein metabolism in starvation depends upon the amount of fat in the body. The calf has little body fat at birth or in the early stages of growth (Armsby & Moulton, 1925), and the effect of starvation is therefore intense. If calves are affected by diarrhoea, and fasting is used as a method of control, it follows a period in which depletion of reserves has already occurred. Realimentation after starvation, if too rapid, may lead to an exacerbation of symptoms, as shown by the behaviour of calf no. 5 during the first period of starvation. Lastly there is no indication that, following realimentation, efficiency of food utilization is enhanced, and the loss in weight, body protein, and body fat can only be restored by establishing a plane of nutrition higher than that before fasting was commenced.

SUMMARY

1. Experiments are described in which two young calves were fasted for periods of 4 days and their metabolism of nitrogen, energy, chloride, potassium, sodium, magnesium, calcium and phosphorus was determined.
2. The mean loss of weight on fasting was 578 g./day. Following the fast, gain in weight, N storage and digestibility of the diet were the same as in the period before the fast.

3. Faeces were produced during fasting in smaller amounts, but of the same dry-matter composition.

4. The loss of urinary N during starvation averaged 250 mg./kg. body-weight/day, a value much greater than in either mature ruminants of the same body size or of the same species.

5. The biological value of the protein was slightly reduced following a fast, but the reduction was not statistically significant. At both levels of feeding, dietary energy supply rather than protein supply was the factor limiting growth.

6. The excretion of urinary metabolites containing N showed that marked increases occurred only in the urea, creatine and uric-acid fractions. Of the increase in excretion over the endogenous metabolism, 94 % was accounted for by excretion of urea, ammonia and creatine. There was no increase in the total excretion of purine N, the small increase in uric acid being compensated for by a fall in allantoin excretion. The distribution of urinary N of the calf during starvation differs from that of the cow, goat or sheep largely because of the increase in the katabolism of body protein.

7. Urinary excretion of sulphur increased, the increase being all in the inorganic fraction, neither the ethereal nor neutral S changing. The ratio N:S of the increased metabolism over the endogenous metabolism indicated that the source of the S was body-muscle protein.

8. There was no acidosis or ketonuria during starvation of the calves. A slight ketosis during realimentation was a constant symptom.

9. Urinary Cl, K, Na and Ca fell during starvation. There was no evidence of bone katabolism during fasting, and the mineral loss could be traced to katabolism of the cell. The losses of Mg and P were smaller than could be accounted for by complete breakdown of the cell, suggesting that nuclear material was not being katabolized in any large quantities.

10. The respiratory exchange was characterized by a constant fall in metabolism throughout the 4 days of the fast. This fall was not affected by the plane of nutrition of the calves, and was three times greater than the slow fall of metabolism that occurs in man with continued fasting. Evidence is produced to show that this fall was not due to failure to reach a postabsorptive state. The fall was at a much greater rate than the weight loss of the calves.

11. The pulse rate fell during starvation and an equation for estimating metabolism from pulse rate is presented. The relation between respiratory rate and minute volume of the respiration is discussed.

12. The loss of weight during fasting was quantitatively accounted for as fat, protein and carbohydrate katabolized and as loss of water from extracellular and intracellular compartments.

Our thanks and appreciation are due to Mr D. Paterson, who was in charge of the care and feeding of the experimental animals and who assisted in the respiratory-exchange determinations, and to Miss C. Sampson and Miss G. Breckenridge for their help with the analyses.

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The Nutrition of the Young Ayrshire Calf

4. Some Factors Affecting the Biological Value of Protein Determined by Nitrogen-Balance Methods

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The biological value of a protein as evaluated by the Thomas-Mitchell procedure (Mitchell, 1923-4) is affected to a considerable extent by the percentage of protein in the diet (Mitchell, 1923-4; Mitchell & Beadles, 1926-7; Hamilton, 1938). When the percentage is high, a larger proportion of the absorbed amino-acids is deaminated, the nitrogen appearing in the urine and the non-nitrogenous moiety ultimately being assimilated as a source of energy. It is only when the protein content of the diet is such that the demand by the tissues for amino-acids is greater than the supply, that maximal biological values are obtained. This inevitably entails partial protein deficiency in the animal and a submaximal rate of growth.

In the diet of the young growing rat the percentage of protein usually adopted for the determination of biological values is 8, whereas in adults percentages as low as 4-5 have to be used to ensure that tissue demand for amino-acids is greater than supply. Similar percentages were adopted in experiments with growing sheep and with growing cattle (Miller & Morrison, 1939, 1942; Harris & Mitchell, 1941; Swanson & Herman, 1943). Experiments designed to study the biological value of dietary protein in the calf during the first few weeks of life do not appear to have been conducted, and

information regarding the protein content of the diet that would render such experiments critical is not available. It is obvious that the very young calf in the first weeks of life is not physiologically comparable to the adolescent rat weighing 60–70 g. as far as protein requirement per kg. body-weight is concerned, especially as it has been shown by Soxhlet (1878) and by Moulton, Trowbridge & Haigh (1923) that early growth in cattle consists almost entirely of deposition of minerals and protein with comparatively little deposition of fat. Preliminary observations on the effect of the level of dried skim milk protein in the diet on its biological value have already been presented (Blaxter & Wood, 1951*b*).

To obtain further information, N balances have been determined on calves given diets containing varying percentages of protein.

EXPERIMENTAL

Diets. The composition of the diets used is summarized in Table 1.

These diets were made twice weekly by methods previously described (Blaxter & Wood, 1951*a*) and were stored at 5°.

Table 1. *Composition of the experimental diets*

Constituent	Diet no.		
	9	11	10
Dried skim milk (g./l.)	83.9	69.7	55.5
Lard, pure (g./l.)	41.8	41.9	42.1
Cod-liver oil (g./l.)	3.3	3.3	3.3
Glucose (g./l.)	—	15.4	30.8
Mineral mixture no. 2* (g./l.)	—	1.7	3.3
Lecithin (g./l.)	—	0.05	0.1
Calculated composition			
Cal./l.	772	744	716
Fat (%)	4.60	4.60	4.60
Protein in dry matter (%)	22.0	18.0	14.0
Protein calories as percentage of total calories	20.5	17.0	13.5

* See Blaxter & Wood (1951*a*)

Experimental animals and methods. Five Ayrshire bull calves were used. At least one N balance was determined on each. These calves were bought from the local market when 1–4 days old and were gradually accustomed to the diet over a period of 10 days before the experiments began. They were fed twice daily at 9 a.m. and at 7.30 p.m. Faeces and urine were collected using the equipment previously described (Blaxter & Wood, 1951*a*).

Dry matter, total N, ash and fat were determined in the faeces, and total N in the urine. The distribution of urinary N was determined in three balance experiments only.

Table 2 summarizes the experiments completed and shows the calves used. The results for the series of thirteen experiments, each lasting 12 days, shown in Table 2 permitted several direct comparisons of one diet with another. Results with very high intakes of diets nos. 9 and 10 were not, however, obtained and the results with diet no. 11 are therefore treated separately.

Table 2. *Arrangement of balance experiments and animals used (calves nos. 7, 8, 9, 11 and 12)*

Diet		
Type and no.	Amount given (l./day)	Calf no.
High-protein 9	2.4	11
	2.6	12
	3.8	7
	4.2	8
Medium-protein 11	3.4	11
	3.8	8
	4.2	9
	6.0	9
	9.0	9
Low-protein 10	2.4	12
	2.6	11
	3.8	9
	4.2	7

RESULTS

Digestibility of the diets. Table 3 summarizes the data obtained on the digestibility of the total dry matter, fat and total N of diet no. 9 (high-protein) and of diet no. 10 (low-protein). The mean digestibility of the dry matter, fat and protein tended to be higher when the diet high in protein was given. Statistical analysis of these results showed, however, that the differences were not significant.

Table 3. *Apparent digestibility for calves of diets nos. 9 and 10 at four levels of intake*

Amount given (l./day)	Dry matter		Total fat		Total N	
	Diet no. 9 (high-protein) (%)	Diet no. 10 (low-protein) (%)	Diet no. 9 (high-protein) (%)	Diet no. 10 (low-protein) (%)	Diet no. 9 (high-protein) (%)	Diet no. 10 (low-protein) (%)
2.4	93.1	86.8	89.6	81.5	87.8	64.6
2.6	95.7	90.8	96.3	86.9	91.3	84.0
3.8	92.9	91.5	92.4	87.7	86.2	82.6
4.2	93.8	95.1	91.9	91.6	91.7	91.5
Mean	93.88	91.05	92.55	86.92	89.25	80.67
Mean difference with its standard error (three degrees of freedom)	2.83 ± 1.73		5.63 ± 2.34		8.58 ± 5.08	

Table 4 summarizes the results obtained when diet no. 11 (medium-protein) was given. It shows that the apparent digestibility of the dietary fat and the apparent digestibility of the total N declined at the lowest levels of intake. This conclusion may also be made from the results with the low-protein diet given in Table 3. As far as the N digestibility is concerned it suggests that a constant fraction of the metabolic faecal N (Schneider, 1935) is present in the faeces of the young calf.

Table 4. *Apparent digestibility for calves of the medium-protein diet no. 11 at five levels of intake*

Amount given (l./day)	Dry matter (%)	Total fat (%)	Total N (%)
3.4	90.9	87.9	86.0
3.8	93.0	86.9	87.2
4.2	97.3	96.7	94.5
6.0	96.6	95.7	93.3
9.0	96.5	95.6	94.1

The percentage of N in the faeces tended to decline with increasing intake, but this was not statistically significant. Differences between the diets in percentage of N in the faecal dry matter were, however, statistically significant. The percentage of N in the dry faeces may be related to the percentage of protein in the diet by the equation

$$N \text{ in dry faeces} = 1.2 + 0.3P,$$

where P = the percentage of the total dietary calories present as protein. The intercept of this equation should represent the percentage N in the dry faeces when there is no N in the diet, that is the metabolic faecal N. The value of 1.2 g./100 g. faecal dry matter differs from the value of 2.0 g. as directly determined (Blaxter & Wood, 1951*a*). The errors involved, especially the assumption of linearity of the regression are, however, large and it is doubtful whether the difference is in fact real. From the variability in the N excretion in the faeces of these calves, ranging from 0.99 g. by calf no. 9 ingesting 4.2 l. of the medium-protein diet no. 11 to 2.73 g. by calf no. 12 ingesting 2.4 l. of the low-protein diet no. 10, it is clear that in the young calf N excretion in the faeces is not constant as in cattle or sheep fed on standard rations. This is largely due to varying degrees of alimentary disturbance in the calves, ranging from acute diarrhoea, when up to 60 % of the ingested N appears in the faeces, to mild digestive upsets, and it would appear that this factor of digestive disturbances is sufficient to prevent the demonstration of even comparatively large differences in the digestibility of diets unless many calves are used.

Urinary nitrogen and nitrogen balance. Table 5 summarizes the N-balance data. These show that for each diet an increase in the amount ingested was associated with very little change in the urinary excretion of N, but with a marked change in N balance. The relation between the intake of N expressed as apparently digested N and the urinary N was examined by analysis of covariance. One observation was omitted, that for the animal in negative balance on the low-protein diet. The analysis of variance is given in Table 6.

There were no statistically significant differences in the slopes of the individual regressions relating urinary N to the N intake apparently digested, but the mean regression was very highly significant statistically. There was a large difference between the urinary N excretion of the calves on the low-, medium- and high-protein diets, at the same level of N intake. The three equations relating urinary N to apparently digestible N were

$$UN_H = 0.21ADN_H + 5.30, \quad (1a)$$

$$UN_M = 0.21ADN_M + 3.71, \quad (1b)$$

$$UN_L = 0.21ADN_L + 2.13, \quad (1c)$$

Table 5. *Nitrogen balances of the calves*

Diet given (l./day)	Intake	Excretion		Balance
		Faeces	Urine	
		Diet no. 9 (high-protein)		
2.4	11.48	1.40	7.63	+2.45
2.6	13.23	1.15	7.85	+4.23
3.4	—	—	—	—
3.8	18.19	2.51	8.46	+7.22
4.2	—	—	—	—
6.0	—	—	—	—
9.0	—	—	—	—
Diet no. 11 (medium-protein)				
2.4	—	—	—	—
2.6	—	—	—	—
3.4	13.77	1.93	6.17	+5.67
3.8	15.48	1.98	7.14	+6.36
4.2	17.80	0.99	6.82	+9.99
6.0	24.18	1.63	8.22	+14.34
9.0	37.68	2.21	11.47	+23.99
Diet no. 10 (low-protein)				
2.4	7.69	2.73	5.76	-0.80
2.6	8.62	1.66	4.46	+2.50
3.4	—	—	—	—
3.8	12.20	2.11	3.97	+6.11
4.2	12.61	1.07	4.61	+6.93
6.0	—	—	—	—
9.0	—	—	—	—

Table 6. *Analysis of variance of the urinary nitrogen excretion of the calves expressed in g./day, including the covariance of urinary nitrogen on apparently digested nitrogen*

Component	Degrees of freedom	Estimated variance	Variance ratio (e^{22})
Joint regression of urinary N on apparently digested N intake	1	17.252	103.7***
Differences between regressions	2	0.035	N.S.
Differences between means	2	7.433	44.6***
Error	5	0.166	—
Total	10	—	—

*** Significant at $P < 0.001$. N.S.: not significant.

where UN represents urinary N excretion in g./day, ADN the apparently digested N and the subscripts H , M and L refer to the high-, medium- and low-protein diets.

The intercepts, 5.30, 3.71 and 2.13, represent the urinary N excretion when no N was given. These values do not necessarily represent the endogenous excretion of N, for in the region of negative N balance the excretion of N would increase, since a low N intake in these experiments must mean a low calorie intake. These regressions therefore may not be comparable to the equations of Allison (Allison & Anderson, 1945; Allison, 1948), based on observations in which adequate caloric intake was in general maintained. As the data used in the present study were all obtained in the region of positive balance, however, it is probable that they represent both an endo-

genous component and a constant 'basal deamination component', that is an amount of urinary N reflecting the higher amount of deamination that occurs on diets high in protein. This quantity should be independent of the dietary source of N.

The N balances were related to the N apparently digested in a similar analysis of variance, presented in Table 7. In this instance the data for the animal in negative balance were included.

Table 7. *Analysis of variance of the nitrogen balances of the calves expressed in g./day, including the covariance of nitrogen balance on the nitrogen apparently digested*

Component	Degrees of freedom	Estimated variance	Variance ratio (e^{22})
Joint regression of N balance on apparently digested N	1	269.253	636.6***
Differences between regressions	2	1.447	3.4 N.S.
Differences between means	2	5.061	12.0*
Error	6	0.423	—
Total	11	—	—

*** Significant at $P < 0.001$. * Significant at $P < 0.05 > 0.01$. N.S.: not significant.

The differences between the individual regressions were not significant but the mean differences between the intercepts of the equation were significant. This is in agreement with the results obtained for urinary N, despite the inclusion of the one value in which the calf was in negative balance. The equations relating N balance to the apparently digested N intake were

$$NB_H = 0.81ADN_H - 5.64, \quad (2a)$$

$$NB_M = 0.81ADN_M - 4.22, \quad (2b)$$

$$NB_L = 0.81ADN_L - 3.13, \quad (2c)$$

where NB represents the N balance in g./day and the other terms have the same significance as for equation (1).

In view of the errors involved in the assumption that the results for an animal in negative balance can be included in the equation, the agreement between the two sets of equations is excellent. When this result is omitted the regression coefficient of N balance on N intake apparently digested is 0.79 and the values for the intercept -5.30 , -3.71 and -2.31 , that is the same as for the equation relating UN to ADN , except that the signs are changed. The equations based on all the data, however, have been used in the ensuing calculations as the justification for discarding the aberrant point cannot be tested at the present time.

From the above equations the results in Table 8 were calculated. They show that if equal quantities of gross energy are supplied, the storage of N falls as the protein content of the diet is reduced. For equal amounts of N apparently digested, however, the storage of N increases with decreasing protein content of the diet. This means that if a protein-free supplement is added to a basal diet, N retention will increase, the effect being to reduce the 'basal deamination component' associated with the higher

Table 8. *Storage of body nitrogen by the calves following ingestion of different amounts of nitrogen in diets with high, low and medium levels of protein (g./day)*

Type of diet	Storage of N when equal quantities of apparently digested N are given		Storage of N when equal quantities of energy are given	
	N given		Gross energy given	
	10 g.	20 g.	2500 Cal.	3500 Cal.
Low-protein	5.00	13.13	4.59	7.68
Medium-protein	4.01	12.04	5.53	9.43
High-protein	2.51	10.64	6.16	10.88

protein content of the basal diet. These relationships can be inferred from the data of Table 5, where comparisons can be made between animals receiving the same quantity of diets high or low in protein.

The body-weight gains of the calves reflect these differences in N retention. Thus, in the three calves given 3.8 l. of one of the three diets, the daily gains in body-weight were 360, 305 and 229 g. for the high-, medium- and low-protein diets respectively. In the calves given 2.6 l. of the high- or the low-protein diet, the daily gains were 155 and 54 g. respectively, whereas with only 2.4 l., the calf on the low-protein diet lost daily 46 g. of weight and the calf given the high-protein diet gained 18 g. For approximately equal intakes of energy, gains were smaller when the diets contained less protein.

A comparison of body-weight gains at equivalent protein intakes irrespective of total calorie intake, can similarly be made. Calf no. 12 ingesting 2.6 l. of diet no. 9 was ingesting about the same quantity of protein as calf no. 11 ingesting 3.4 l. of diet no. 11 and calf no. 9 ingesting 3.8 l. of diet no. 10. The daily gains in weight were 155, 275 and 304 g. respectively. Similarly, the calf ingesting 3.8 l. of diet no. 9, gained 360 g. and the calf ingesting 4.2 l. of diet no. 11 gained 455 g. The intake of N of both animals was about the same. The body-weight gains, however, are subject to greater errors of estimation than are the N balances.

Biological values of the ingested protein. The biological value of a protein, as defined by Mitchell (1923-4), is given by the equation

$$BV = 100 \times \frac{NI - (UN - EN) - (FN - MN)}{NI - (FN - MN)}, \quad (3)$$

where NI = nitrogen intake, UN = urinary N excretion, EN = endogenous N excretion, FN = faecal N excretion, MN = metabolic component of the faecal N, BV = biological value. This equation may be rearranged by substituting the nitrogen balance (NB) for the necessary terms in the numerator of the equation and replacing the term $(NI - FN)$ by the term ADN (apparently digested N). This gives the modification

$$BV = 100 \times \frac{EN + MN + NB}{ADN + MN} \quad (3a)$$

Now it has already been shown that the N balance may be related to the amount of apparently digested N by a simple linear equation of the type used by Allison (1948) (see equations (2a), (2b) and (2c)). The biological value as defined by Mitchell (1923-4) can thus be determined by substituting in the above equation the linear equation

relating ADN to NB . It is also necessary to include values for excretion of endogenous N and of metabolic faecal N . These may, for the moment, be regarded as constants of 2.5 and 0.6 g. N respectively.

The biological value of the proteins of dried skim milk can thus be estimated from the equation

$$BV = 100 \times \left(\frac{2.5 + 0.6 + (0.81ADN - x)}{ADN + 0.6} \right), \quad (4)$$

where x represents the intercept of the N -balance equation on the apparently digested N axis.

From this equation it is clear that the magnitude of the biological value obtained will depend on the value of this intercept; it will be greater when the intercept is small, as with the low-protein diets. It will also be determined by the amount of apparently digested N taken in, the biological value being greater the more N is apparently digested. This relationship, however, can only apply if the equation relating NB to ADN is linear. Little is known at the moment regarding the linearity of this equation at high levels of intake, but it has recently been shown by Blaxter & Wood (1951c) that linearity of the equation still applies when sufficient whole milk is given to result in N balances of up to 25 g./day, that is equivalent to gains in body-weight of 910 g. and to an energy intake about 2.5 times the maintenance requirement.

The 'biological value' of a protein in an animal with a high capacity for N retention is thus a simple inverse function of the amount of diet consumed and a linear function of the intercept of the N -balance equation of Allison (1948). This in turn implies that the regression coefficient in the N -balance equation represents the approximate biological value attainable, expressed as a decimal. In fact it is too low a value, since the inclusion of the metabolic faecal N in equation (4) would increase the asymptote slightly.

Table 9. *Mean biological values of skim milk protein for calves determined by nitrogen-balance methods*

Amount of diet given (l./day)	Biological value		
	High- protein diet (%)	Medium- protein diet (%)	Low- protein diet (%)
2.4	40.0	—	51.2
2.6	54.8	—	61.6
3.4	—	74.5	—
3.8	64.6	68.1	87.9
4.2	—	78.0	80.0
6.0	—	80.2	—
9.0	—	80.2	—

It may be expected, therefore, that the biological values determined in the present experiments would be in agreement with the hypothesis presented above, and this is in fact shown in Table 9. For the high-protein diet, the biological values increased rapidly as the amount of diet given was increased from that allowing a daily gain of only a few g. to a quantity permitting a gain of 360 g. A similar increase is shown as well

for the diets of medium and of low-protein content. Errors are, however, attached to these estimates, and the values for metabolic faecal N and the endogenous excretion of N vary from animal to animal. Both quantities were estimated using the relationships between basal heat loss and endogenous metabolism, and faecal dry-matter excretion and metabolic N excretion (Blaxter & Wood 1951*a*).

DISCUSSION

It is apparent that the biological value of a protein estimated by N-balance methods is by no means a constant. This statement, however, ignores one salient fact, that in order to measure the biological value of a protein, it is essential that the animal should not deaminate amino-acids to use the carbon-containing moiety as a source of energy in maintenance or in growth. A biological value, if it is to be a criterion of the balance of essential amino-acids making up a protein, must be determined under conditions whereby such a need for energy does not arise. Thus, despite the variability of the biological value, a valid estimate may be obtained under conditions where the possibility of deamination of the constituent amino-acids of a protein to provide energy is specifically excluded. It follows, however, that the use of diets of constant protein content would not result in comparable estimates of biological value. A low intake of the diet would depress this estimate, and the depression would be greater the higher the protein content of the diet.

The reason for this relationship between level of feeding and the utilization of dietary protein is to be found in the relative amounts of protein and energy needed for the growth and the maintenance of the animal. It is obvious that if the relative requirements for protein and energy are not the same for maintenance and for growth, then the requirement of a growing animal for protein will not be constant in relation to its energy requirement, but will vary according to the amount of growth. To study this aspect of the problem, the results from N-balance experiments have been analysed statistically.

First, the relation between the N balance of the young calf and its gain in body-weight was determined in N-balance experiments conducted in this laboratory. This information is shown in Fig. 1, and analysis of variance showed that the regression of N balance on body-weight was highly significant statistically ($P < 0.001$). The equation of the regression was:

$$NB = 0.0262G + 0.703, \quad (5)$$

where NB is the N balance in g./day and G the gain in weight in g./day. The intercept of 0.7 g. N, although only significantly different from zero at odds of 11:1, implies, that when there is no gain in weight storage of N still takes place, that is body-weight maintenance does not entail cessation of growth.

The regression coefficient of 0.0262 shows that for every gain of 100 g. body-weight the calf stores 2.62 g. N, or 16.5 g. protein. This may be interpreted to mean that the major part of the gain in body-weight in the young calf is a gain of flesh, for 16.5 g. protein would be expected to be present in about 80–85 g. muscle. The remainder must represent fat, bone and water. The energy value of the gain in body-weight can only be determined calorimetrically or by analysis of the carcass, but a first approxi-

mation can be made by statistical analysis of results obtained with diets containing ample protein and relating the digested energy intake to the gain in body-weight. The regression of body-weight gain (*G*) on calorie intake was found to be

$$G = 0.326 DC - 577.5,$$
 (6)

where *DC* represents the total number of Cal. ingested daily less the Cal. excreted in the faeces.

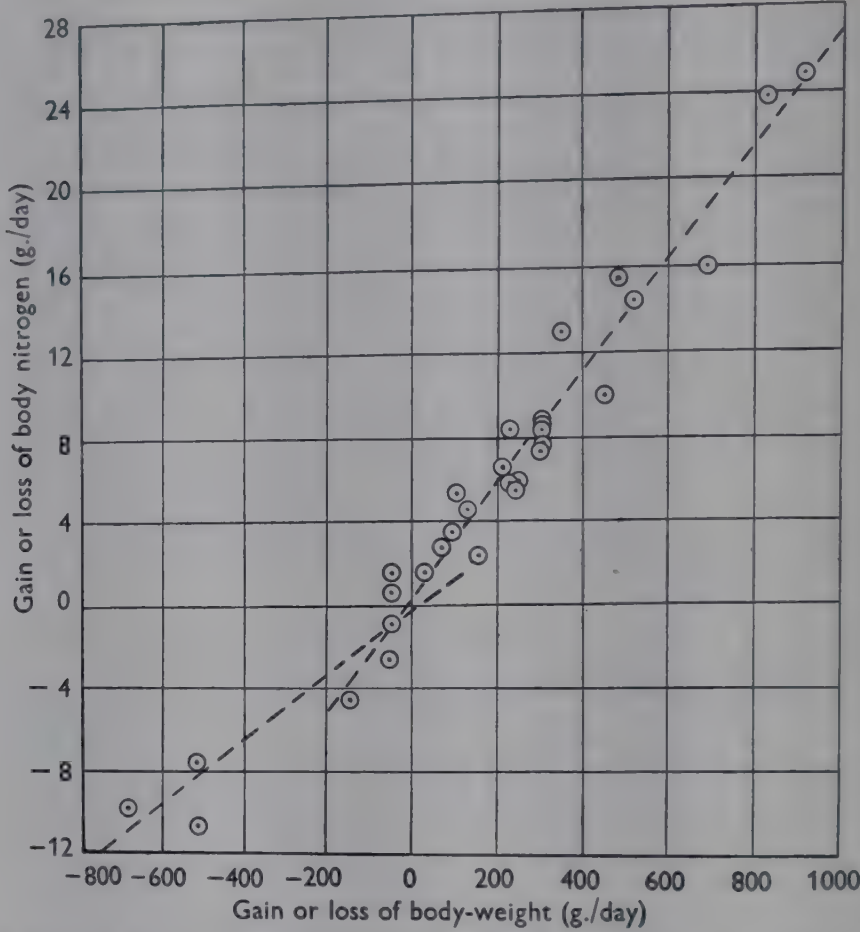


Fig. 1. Relation between body-weight gain or loss of calves and their gain or loss of body nitrogen.

Table 10. *Net protein requirements, uncorrected for losses in digestion or in metabolism, and digestible energy requirements of young calves weighing 30 kg., together with an estimate of the percentage of the total calories that must be present as protein in order to avoid deamination of dietary protein to meet energy requirements*

Gain in weight (g./day)	N retention (g./day)	Endogenous N (g./day)*	Total protein required by the tissues (g./day)	Digestible energy required (Cal./day)†	Percentage of the digestible energy needed as protein to avoid deamination
0	0.70	2.40	19.4	1572	6.9
100	3.32	2.40	35.7	1879	10.7
200	5.94	2.40	52.1	2186	13.5
400	11.18	2.40	84.9	2800	17.1
800	21.66	2.40	150.4	4028	21.1
1000	26.90	2.40	183.1	4642	22.2

* 80 mg./kg. body-weight.
† 52.4 Cal./kg. body-weight + 307 Cal./100 g. gain in weight.

This relationship was highly significant statistically, though, as may be inferred from Fig. 2 which shows the relation between gain in body-weight and the energy intake expressed per kg. body-weight, the variation about the regression was high. This variability is understandable since the errors involved in determining gain in weight and caloric intake are high; no account is taken of the loss of energy in the urine for diets high in protein or of the variation from animal to animal in energy requirements for maintenance or growth. The equation is only a generalization but it is probable that it represents the average actual relationship.

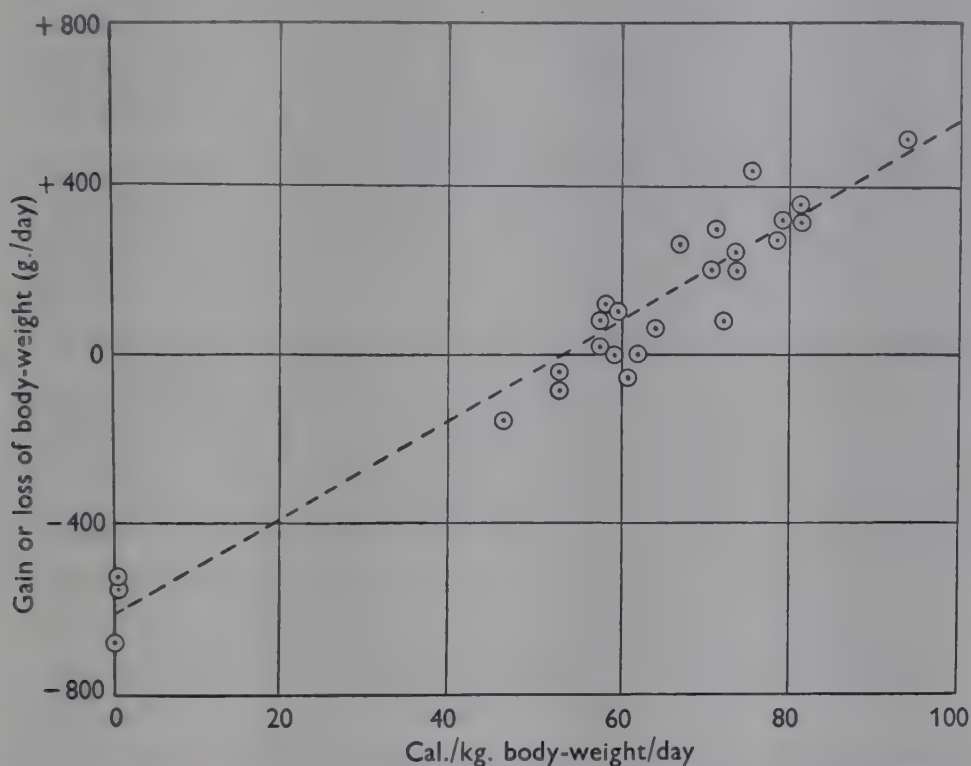


Fig. 2. Relation between the energy ingested by calves less that in the faeces, and the daily gain in body-weight.

The constants of the equation may be interpreted to show that in fasting the daily body loss is approximately 580 g. and that every additional Cal. is associated with a gain of 0.326 g. body-weight. By simple arithmetic it may be calculated that the calorie intake when there is no change in body-weight is 1771 Cal. and that each gain of 100 g. body-weight is associated with an intake of an additional 307 Cal. The former may be interpreted to represent the requirement of energy for body-weight maintenance, and the latter the requirement for body-weight gain. That these are essentially correct estimates is shown by the fact that the maintenance requirement per kg. body-weight would be 52.4 Cal. of 'digested' energy, a value in excess of the determined basal metabolism of 40–43 Cal./kg./day (Blaxter & Wood, 1951*a*) by an amount of energy that may be accounted for by an activity increment of 25–30 %. Such an activity increment is extremely likely for an animal confined in a metabolism cage (Blaxter, 1948). Similarly, the value of 307 Cal. for the calorific value of the digested nutrients associated with 100 g. gain in body-weight is higher than that of the gain itself, as judged by comparison with the carcass analyses of Armsby & Moulton (1925).

It has previously been shown (Blaxter & Wood, 1951*a*) that the endogenous N

metabolism of the calf is 80 mg./kg. body-weight. This value represents the animal's minimum requirement of N for maintenance of N equilibrium. From it, together with the results given in equations (5) and (6), an estimate of the requirements of protein and of digestible energy can be made. The protein requirements are net requirements in the sense that they represent only the amounts of protein which are stored or which are equivalent to the endogenous metabolism (Blaxter & Mitchell, 1948). With proteins of 100 % biological value and 100 % digestibility they represent minimal requirements of dietary protein that ensure no deamination of protein for energy purposes (Blaxter & Mitchell, 1948).

The results of the calculation are given in Table 10. The last column of the table shows that the percentage of the digested energy that must be present as protein increases with increasing rate of gain. These figures apply to proteins of 100 % biological value only; they would be higher if the biological value of the protein was less than 100. If an animal were given a diet containing 20 % of its digested energy as a high-quality protein, maximal biological values would not be attained unless the total intake was sufficient to result in a gain of at least 700 g./day. If the intake was smaller, the urinary excretion of N would relatively increase, since the protein given would be greatly in excess of the requirements at that particular rate of gain in weight.

The relation between the amount of N absorbed and the body balance of N has recently been studied extensively (Melnick & Cowgill, 1937; Harris & Mitchell, 1941; Allison & Anderson, 1945; Bricker, Mitchell & Kinsman, 1945; Hegsted, Tsongas, Abbott & Stare, 1946; Bricker & Mitchell, 1947; Barnes, Bates & Maack, 1946). Most, but not all, of these experiments were concerned with N balances below maintenance, and adequate energy was supplied. The experiments with growing rats with energy intakes above maintenance were mostly carried out, however, with diets containing a constant percentage of protein. Allison (1948) recently reviewed this work and concluded that the nitrogen-balance index (Allison, Anderson & Seeley, 1946) is 'some function of but not necessarily equal to the biological value of the protein source'. This nitrogen-balance index is not exactly equivalent to the regression coefficients of our equations (2a), (2b) and (2c), as it differs in the use of apparently digested N, and not truly digested N, as the independent variable. The meaning of this regression coefficient is, however, little altered, and it would appear that it does, in fact, represent the maximal biological value of the protein. That this relationship applies to intakes above maintenance in the calf is merely a reflexion of the intensity of its N metabolism, for in the young animal there is no indication of a limit to the capacity to store N at least up to a body-weight gain of about 1 kg./day. In mature animals the relationship of N balance to N intake is curvilinear above maintenance, because the capacity of the animal to store N is limited.

SUMMARY

1. Experiments in which five calves were given varying quantities of semi-synthetic, liquid diets containing high, medium and low percentages of dried skim milk protein are described.
2. The digestibility of the dry matter, fat and nitrogen tended to be lower when

low-protein diets were given, and the percentage of N in the dry faeces was much higher for the animals given the highest level of protein.

3. The urinary N increased at a constant rate with increasing intake of apparently digested N, irrespective of the amount of protein in the diet. The excretion of urinary N at any one level of intake was greatest for the diet containing the most protein.

4. The N balance also increased at a constant rate with increasing intake of apparently digested N, and the slope was independent of the N content of the diet. At comparable intakes of apparently digested N, storage was greater on the diet containing the smaller percentage of protein. At comparable intakes of energy, however, storage of N was greater for the diet high in protein.

5. The biological value of ingested protein was shown to be a simple inverse function of the amount of dietary N apparently digested and a linear function of the intercept of the equation relating N balance to apparently digested N, the magnitude of the intercept depending on the percentage of protein in the diet.

6. The slope of the regression of N balance on apparently digested N for a diet of constant composition appeared to be an approximation to the maximal biological value of the protein. This slope was comparable to the N-balance coefficient of Allison (1948). The biological value of the dried-milk proteins was thus found to be 81.

7. The reasons for these relationships are discussed, and it is pointed out that if the biological value of a food protein is defined as a description of N metabolism when there is no deamination of ingested amino-acids for provision of an energy source, then cognizance must be taken of the fact that the protein required is not a constant percentage of the energy requirement, but increases as the rate of growth increases. If diets of constant composition are used, they must be given at a level that ensures an excess of dietary energy.

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The Growth Response of Rats to Purified Diets

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In attempts to design a complete purified diet some observations were made on the rates of weight increase in rats receiving simple basal diets with various supplements. A recent review by Dunn, Murphy & Rockland (1947) on the optimal growth rate of the rat increased our interest in the studies of Zucker, Hall, Young & Zucker (1941 *a-c*) on the relation of weight to age in the rat and, although the numbers of rats in our groups were small, it appeared to be worth recording their growth performances for the information of others who may be concerned with various problems involved.

EXPERIMENTAL

Animals. The animals were rats of the black-and-white Lister Institute stock taken at weaning, aged 21–23 days and weighing 35–45 g. They were placed immediately in open-grid cages of the type used for experiments on vitamins of the B complex and were given experimental diets, with various supplements, from 28 days of age. During the 1st week there were four animals in a cage; thereafter they were housed separately. Litters of eight rats were used, and in all experiments diets, doses and supplements were distributed in such a way as to give the fullest possible litter-mate comparison with due regard to sex.

Diets. The diets used in all tests were based on the following formula expressed as percentages: casein 20, sucrose or maize starch 60, hardened arachis oil 12, lard 3, salt mixture (McCollum 185) 5. When sucrose was the source of carbohydrate the diets were given dry and uncooked; when starch was used the dry ingredients were mixed with an equal weight of water and steamed until the starch was completely cooked, in order to obviate refection (Fridericia, Freudenthal, Gudjonsson, Johansen & Schoubye, 1927). The cooked diets are particularly convenient because they can be given as solid blocks that are not easily wasted in open-grid cages. In the earlier tests vitamins A and D were given as 0.02 ml. cod-liver oil daily; later Adexolin (Glaxo Laboratories Ltd.) was diluted with arachis oil so that one drop once a week provided 120 i.u. vitamin A and 20 i.u. vitamin D. In these tests vitamin E was given also as a weekly dose of 1 mg. α -tocopherol acetate dissolved in arachis oil and always at least 48 hr. after the dose of vitamins A and D.

The members of the vitamin B complex now obtainable as pure crystalline substances were given as a solution containing in 1 ml.:

Vitamin B ₁	10 μ g.	Riboflavin	40 μ g.
Vitamin B ₆	10 μ g.	Nicotinic acid	1 mg.
Pantothenic acid	100 μ g.	Biotin	0.2 μ g.
<i>p</i> -Aminobenzoic acid	1 mg.	Folic acid	2 μ g.
Inositol	1 mg.	Choline	3 mg.

A daily dose of 1 ml. of this solution provided an adequate supply of all the B-vitamins, since no beneficial effect was observed if it was increased.

The aqueous liver extracts used included a crude commercial extract of whole liver and various filtrates obtained after whole-liver extracts had been treated with charcoal. All the extracts were supplied by Glaxo Laboratories Ltd. with data as to the equivalence of the extracts in terms of the weight of liver from which they had been made. The daily doses of liver extract were material from 6 g. original liver.

Three different types of casein were used. They were an alkaline caseinate (of the 'light white casein' type) from Glaxo Laboratories Ltd., a highly purified 'Labco' casein from the Borden Company, U.S.A., and a casein prepared in New Zealand by acid washing but without extraction by organic solvents.

To some animals a diet containing 15 % yeast was given. The yeast was a commercial dried brewer's yeast and replaced 15 % of starch in the diet. In all tests some rats were given the normal stock diet, which consisted of a mixture of full-cream dried milk, bread, whole wheat, bran, wheat germ, oat flakes, fishmeal and meat. These animals were kept in separate cages and otherwise treated exactly like those having the synthetic diets.

Experimental arrangement. For the final experiments the numbers of rats, the diets and the doses in the different groups were:

- A. Sixteen rats on Labco casein diet and solution of B-vitamins.
- B. Sixteen rats on Labco casein diet, solution of B-vitamins and a charcoal filtrate from liver.
- C. Sixteen rats on 'light white casein' diet and solution of B-vitamins.
- D. Sixteen rats on 'light white casein' diet, solution of B-vitamins and charcoal filtrate from liver.
- E. Eight rats on 'light white casein' diet containing 15 % dried yeast.
- F. Eight rats on New Zealand casein diet and solution of B-vitamins.
- G. Eight rats on New Zealand casein diet, solution of B-vitamins and charcoal filtrate from liver.
- H. Fourteen rats on normal stock diet.

RESULTS

Preliminary tests indicated that a satisfactory weight increase could be obtained in rats on a diet of highly purified materials with all the known vitamins in crystalline form, but that the weight increase was not as great as that obtained in comparable animals receiving a liver extract. It was established also that a charcoal filtrate from whole liver supplemented with the solution of B-vitamins gave the same weight increases as crude liver extracts. In all subsequent experiments, therefore, the charcoal filtrate was used, being more easily obtainable. These tests suggested that the liver extracts contained some factor or factors different from any of the individual vitamins used.

The results of the final tests with eight different groups of rats are summarized in Table 1, showing the average weekly weight increases of the males and females for a period of 14 weeks. The growth performance of the males was, as usual, always superior to that of the females. The differences between groups with and without liver

Table 1. *Average weekly growth responses of rats during 14 weeks on different diets*

Group	Diet	No.	Sex	Mean weekly weight increase with its standard error (g.)
A	Labco casein and solution of B-vitamins	8	♂	16.4 ± 0.4
		8	♀	11.6 ± 0.4
B	Labco casein, solution of B-vitamins and liver extract	7	♂	17.7 ± 0.7
		9	♀	11.7 ± 0.4
C	Light white casein and solution of B-vitamins	8	♂	16.3 ± 0.1
		8	♀	10.7 ± 0.4
D	Light white casein, solution of B-vitamins and liver extract	7	♂	17.5 ± 1.0
		9	♀	11.8 ± 0.6
E	Light white casein and 15 % yeast	4	♂	18.8 ± 0.9
		4	♀	10.7 ± 0.4
F	New Zealand casein and solution of B-vitamins	4	♂	16.2 ± 0.5
		4	♀	11.5 ± 0.3
G	New Zealand casein, solution of B-vitamins and liver extract	4	♂	18.5 ± 0.8
		4	♀	12.3 ± 0.7
H	Normal stock diet	6	♂	21.8 ± 0.5
		8	♀	13.6 ± 0.7

extract and between those with purified and stock diets were examined statistically by the *t* test and were mostly not significant. This was probably due to the small numbers in each group. No group having purified diet even with the supplement of liver extract showed mean weight increases equal to those of rats having the normal stock diet. The close approximation of the growth of the animals in all groups, except those on stock diet, is shown in Fig. 1*a, b*, which are the curves of mean weight increase plotted against age in weeks.

DISCUSSION

During 1941 Zucker *et al.* (1941*a-c*) published a series of papers on the growth of the rat, and their observations on the relation of weight to age in rats receiving adequate 'normal' diets led them to state that growth might be represented by a straight line when the logarithm of the weight was plotted against the logarithm of the age in days from the time of conception up to 28 days after birth, and that thereafter a departure from this straight line occurred and the linear relationship was then obtained by plotting the logarithm of the weight against the reciprocal of the time. The formula on which this line was based expresses the relationship of growth rate to age and can be expressed algebraically as

$$\log W = -k/t + \log A,$$

W being the weight at time *t*, *A* the maximum weight approached asymptotically in the adult animal, $\log A$ the intercept of the straight line and *k* the slope of the line, which characterizes growth rate. The value for *k* was obtained from the formula

$$k = \frac{\log W_2 - \log W_1}{1/t_1 - 1/t_2}.$$

From Zucker's studies of his own rat colony and from calculations made on data from others a series of lines was obtained, each slope being characteristic of a particular colony. The slopes of the lines for females were always flatter than those for males and the values for k tended to be more variable for females.

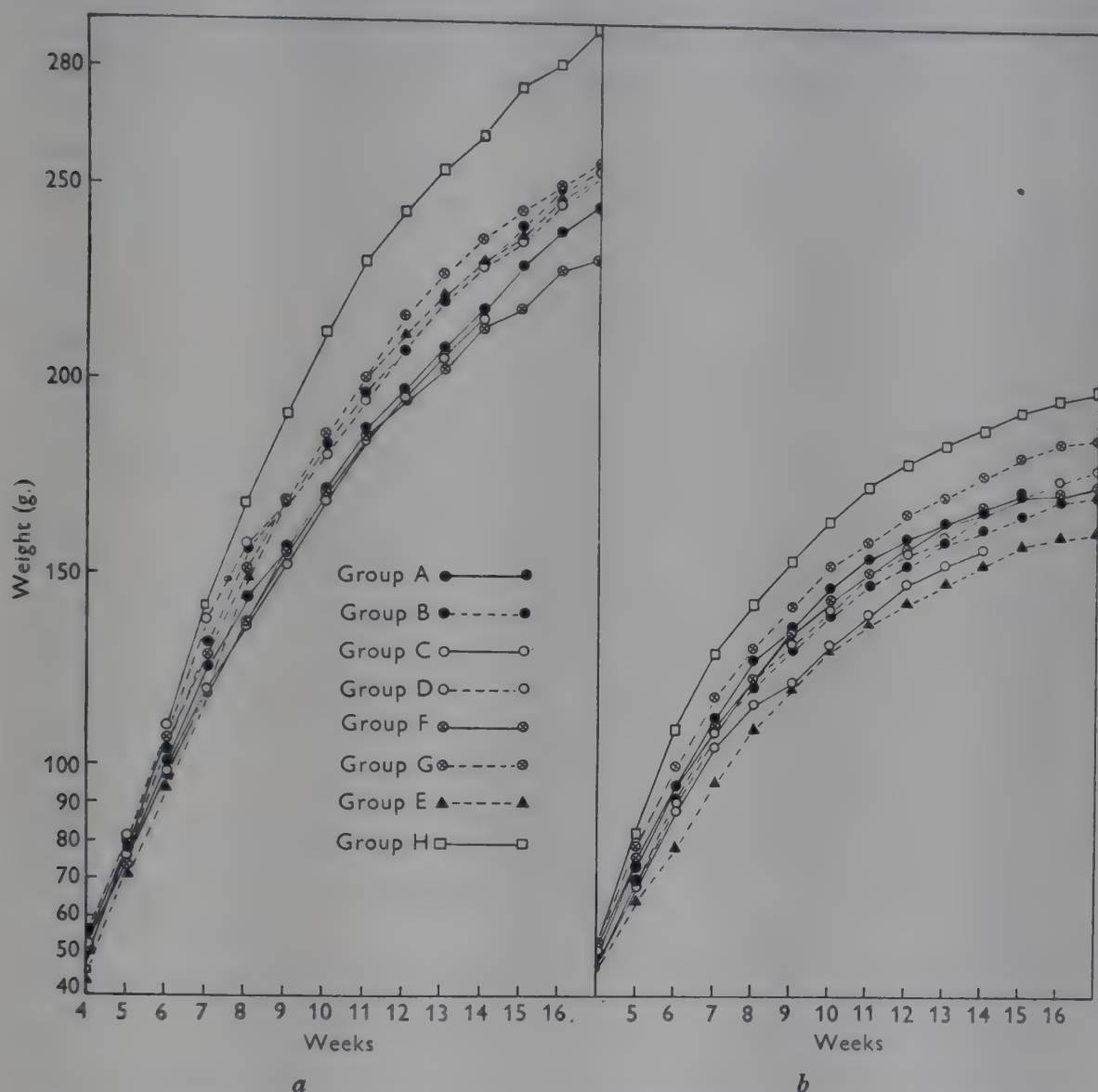


Fig. 1a. Curves showing the relation between age in weeks and mean body-weight in g. of male rats on various diets.

Fig. 1b. Curves showing the relation between age in weeks and mean body-weight in g. of female rats on various diets.

Zucker & Zucker (1943) showed that departure from the straight line expressed by $\log W = -k/t + \log A$ indicated inadequacy in the diet.

In view of these reports it seemed useful to apply Zucker's method of calculation to the results of the growth tests with the synthetic diets, although the numbers of animals in our groups were small in comparison with those used by Zucker. The curves obtained by his formula are set out in Figs. 2 and 3; it will be seen that, in spite of the limitations of the tests, reasonably straight lines are obtained at first for all the different diets. After some weeks the points tend to fall away from the lines in those groups in which the rats had the diet containing 15 % yeast or the stock diet. In these two sets

of curves, both for males and females, it seems that there is a deflexion of the line at about 11 weeks of age. A similar deflexion of the growth curve was found by Dunn *et al.* (1947) in rats of the Long-Evans strain receiving the diet of Anderson & Smith (1932), which was rich in protein. This suggests that the diet containing 15 % yeast and the normal stock diet probably provided more than adequate protein which might be expected, in accordance with the findings of Anderson & Smith (1932) and Dunn *et al.* (1947), to hasten early growth and to cause a subsequent decline in growth rate.

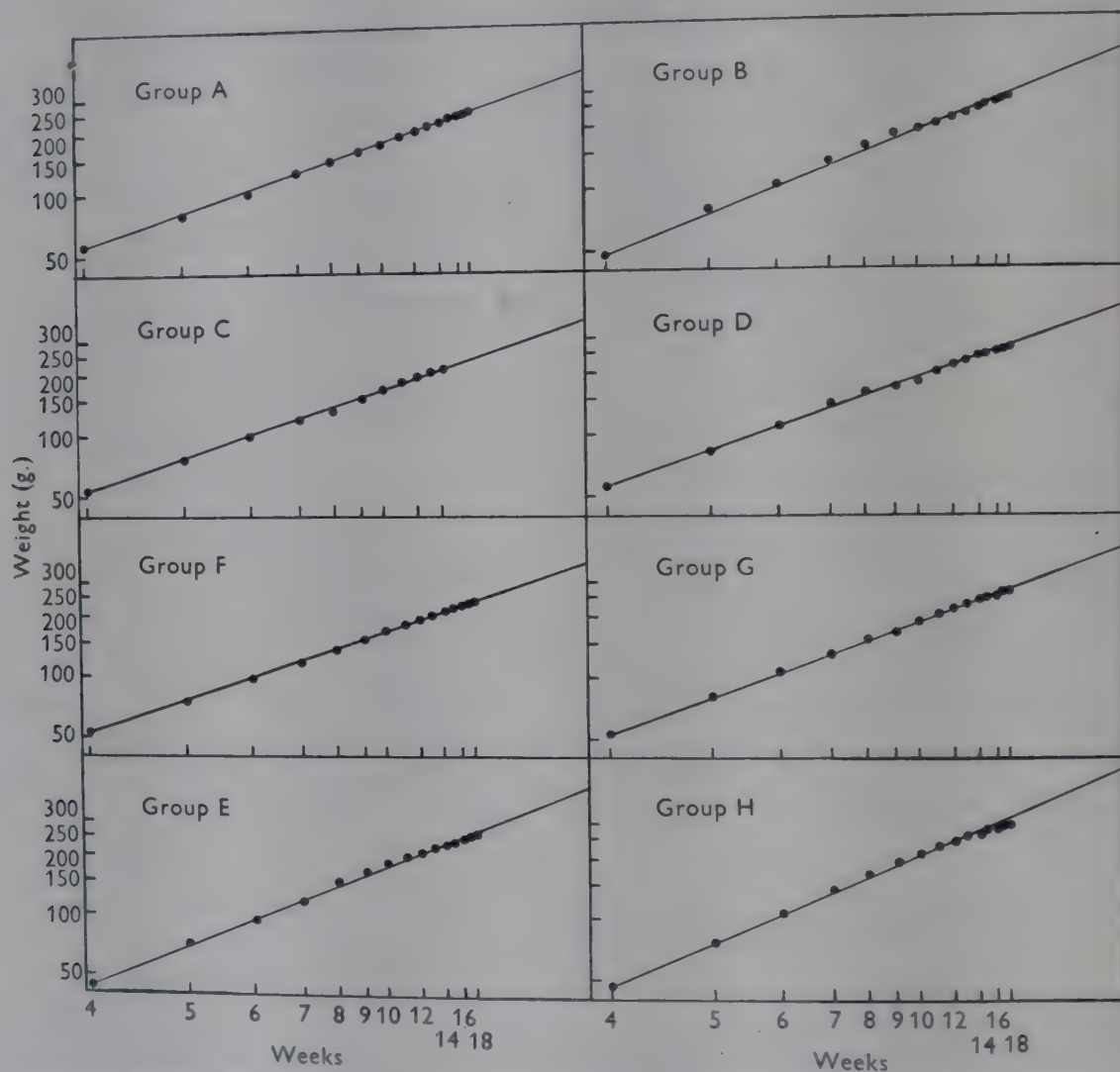


Fig. 2. Curves showing the relation between the reciprocal of the age in weeks and the logarithm of the mean body-weight in g. of male rats on various diets.

This may be contrasted with observations of Zucker & Zucker (1943) on the deflexion of the growth curve in the early stages of growth with a diet containing inadequate protein. The stimulating effect of including 15 % of yeast in the diet was more marked in males than in females. This was of interest in view of the observation of Zucker & Zucker (1944) that males require more protein than females. It has recently been suggested by Gray & Addis (1948) that Zucker's curves may be used to test for abnormal growth of a rat colony. The results obtained in the present study confirm the value of the method for demonstrating normality of growth responses within a particular colony.

In all the curves of the groups other than those having yeast or stock diet, the points tend to fall very close to the straight line. For the females there are more irregularities,

but the general trend of the curves encourages the view that the synthetic diets, even without the addition of liver extracts, are fairly satisfactory for normal growth.

The small increases in growth obtained when liver extract was added to the diets, as shown in Table 1, would seem to indicate that some essential factors were lacking in a purified diet supplemented with the known synthetic vitamins. The fact that liver extracts can provide a further growth stimulus with these diets seems to suggest that at least one factor may be the 'animal protein factor', zoopherin, described by Zucker, Zucker, Babcock & Hollister (1948).

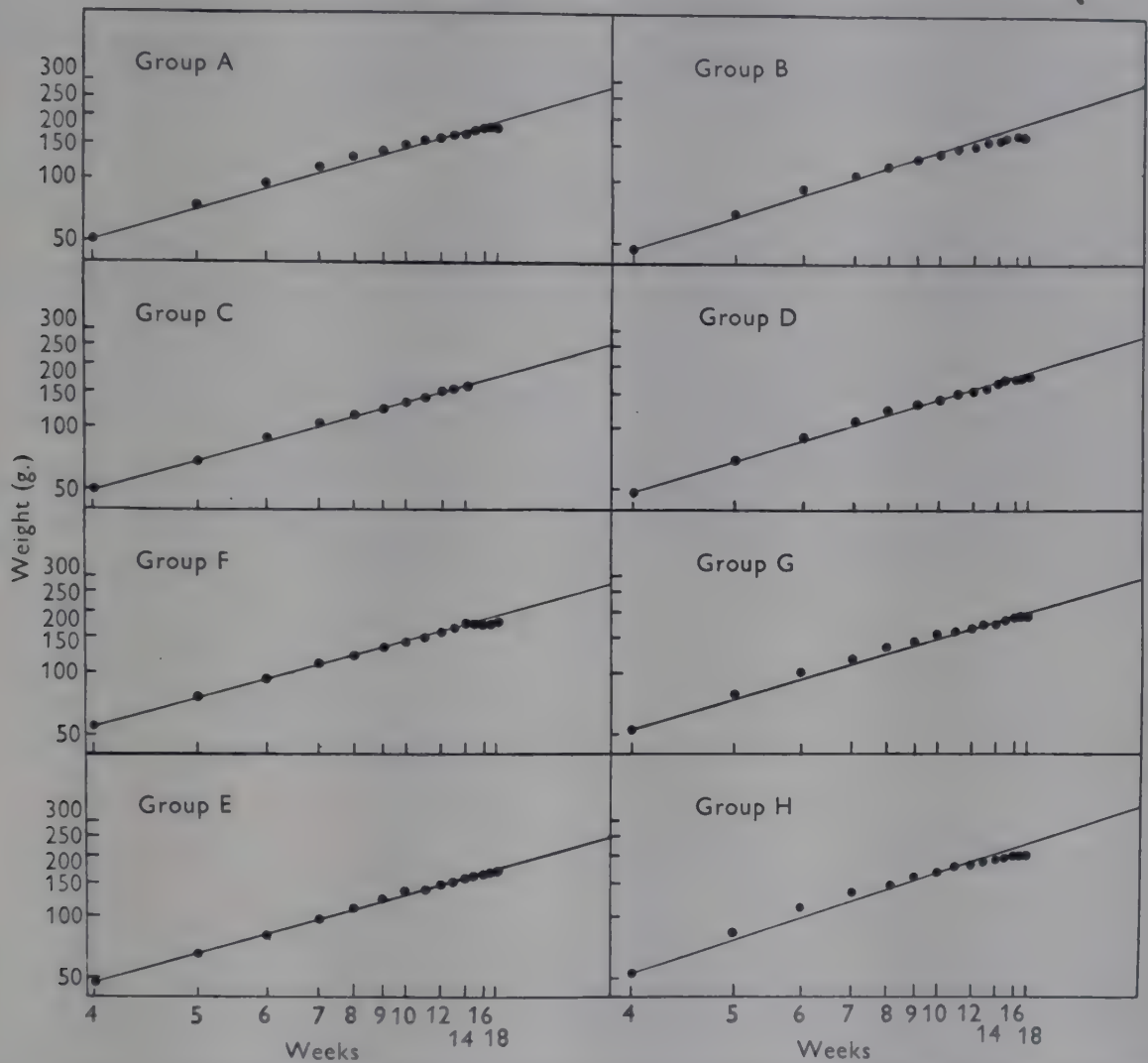


Fig. 3. Curves showing the relation between the reciprocal of the age in weeks and the logarithm of the mean body-weight in g. of female rats on various diets.

SUMMARY

1. The rate of weight increase was studied in young rats receiving purified diets supplemented with synthetic vitamins with and without addition of liver extract.
2. Studies were made also with a diet containing 15 % dried yeast and with a normal stock diet.
3. Addition of liver extract to a highly purified diet gave a slightly increased growth rate, but even with added liver extract the growth rates of animals having purified diets were not equal to those of rats on normal stock diet.

4. The results are shown in curves of mean weight increase plotted directly against age and also according to Zucker's method for obtaining straight-line curves.

5. The results appear to confirm the observations of other workers that Zucker's curves provide valuable information about the normality of growth response within any given rat colony.

We acknowledge our indebtedness to Sir Charles Martin for many stimulating discussions that led to this investigation, and we thank him and Dame Harriette Chick for their continued helpful advice throughout the work. We thank also the Accessory Food Factors Committee of the Medical Research Council for samples of Labco and New Zealand casein, Messrs Glaxo Laboratories Ltd. for the liver extracts, and Messrs Roche Products Ltd. for making up special tocopherol solutions.

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The Estimation of Glucose-Containing Substances in Micro-Organisms from the Rumen of the Sheep

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Before studying glucose-containing substances in the micro-organisms from the rumen of sheep fed on hay, it was necessary to develop a method of estimation specific for glucose. Methods for the estimation of reducing sugars depend largely either on direct determinations of the reducing values of solutions or on such determinations before and after treatment of the solutions with bacteria or yeasts specifically fermenting single sugars or a group of sugars. Since such values are usually expressed in terms of an arbitrarily chosen sugar, the errors resulting when mixtures of reducing sugars are present, or when a large amount of non-sugar reducing material is present, can be considerable. In addition, the interpretation of the value of the non-sugar reducing materials is rendered difficult because different values are obtained depending on the method of estimation employed.

The procedure which has been devised involves quantitative paper-partition chromatography (Flood, Hirst & Jones, 1947) of hydrolysates of the rumen micro-organisms. The results of this method have been compared with the loss in reducing substances in the hydrolysate after fermentation with baker's yeast (The United Yeast Co. Ltd. (D.C.L.), King Street, Aberdeen).

EXPERIMENTAL AND RESULTS

Paper chromatographic method

Apparatus. All-glass tanks and troughs were used, since quantitative recovery of glucose was not obtained with a stainless steel trough. The paper used was Whatman no. 54, since on this paper the sugars are maintained as compact 'spots' and a more regular solvent front is obtained than with the coarser-fibred Whatman no. 1 paper.

Since complete extraction was not always obtained by using the extractor originally described by Flood *et al.* (1947) the all-glass extractor shown in Fig. 1 was devised. This type of extractor was found highly satisfactory. It permits total extraction in 20-30 min.

Chromatographic technique. The method used was essentially that described by Flood *et al.* (1947) as modified by Jermyn & Isherwood (1949). A horizontal micro-capillary burette was used to apply the solution to the paper.

The sheets were run in a mixture containing *n*-butanol (40 parts), acetic acid (10 parts) and water (50 parts) for 24-48 hr. (Partridge, 1948), after which they were dried at 105° and the 'side-strips' removed and sprayed as described by Flood *et al.* (1947). In all these operations forceps were used to handle the cut paper strips. The sections

of the paper horizontally adjacent to the located glucose spots were then removed, rolled with forceps and placed in the cup of the extractor. The extractor was fitted to a ground-joint boiling tube, and 10 ml. distilled water were delivered through the paper roll into

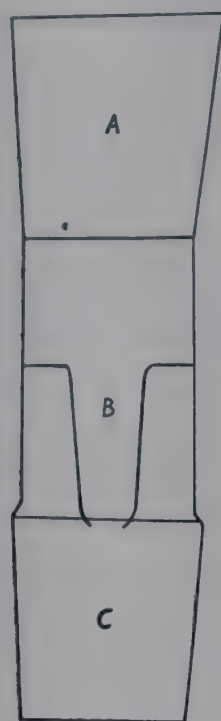


Fig. 1. Paper-strip extraction apparatus. *A* and *C* are ground-glass joints. *B* is a glass cup to contain the paper strip. The cut paper strip is rolled and the roll placed in the inner cup, *B*. The extractor is connected to a condenser at joint *A* and to the extraction tube, containing water, at *C*.

the tube. The tube and extractor were then fitted to a reflux condenser and heated so that the condensate bubbled about the paper roll. At intervals the heater was removed and the condensate allowed to drain into the tube. The tubes were cooled, 3 ml. water added through the paper roll and the extractors removed. The reducing sugars in the extracts were estimated by the method of Hagedorn & Jensen (1923) using sodium starch glycollate as the indicator in the titration (Peat, Bourne & Thrower, 1947).

Before each estimation the extractors and tubes were cleaned in hot chromic-sulphuric acid to prevent interference from grease. With this procedure, the blank values of the paper strips were 1.85 ± 0.04 ml. 0.005 N-sodium thiosulphate, and the recovery of glucose added to the strips ranged from 94 to 100 %.

Preparation of hydrolysates for chromatography. Either freshly centrifuged rumen micro-organisms or acetone powders were used. The acetone powders were prepared by blowing a thick suspension of the micro-organisms from a pipette into 10 vol. ice-cold acetone, the mixture being stirred rapidly during this addition. The suspension was immediately filtered, washed with a little ether, and dried in vacuo at room temperature. The powders were hydrolysed in sealed tubes with N-HCl at 100° for 3 hr. The seals were then broken and the contents of the tubes were adjusted (Congo red) to pH 4.5 with silver

carbonate. After centrifuging, portions of the supernatant liquids were evaporated to dryness at room temperature over phosphorus pentoxide under reduced pressure. The residues were taken up in either 0.5 or 1.0 ml. distilled water and centrifuged to remove solid particles. The clear supernatant liquids were added to the paper from a micro-burette. The concentrations of these solutions were adjusted so that about 200–250 μ g. glucose were added to the paper.

Notes on the procedure. The blank strips were cut from above the point of application of the solution to eliminate any possible interference from non-sugar contaminants. The hydrolysates of the micro-organisms contained many substances that fluoresced under an ultraviolet lamp. A preliminary examination of the dried, developed sheet indicated the shape of the solvent 'front' which had passed out of the paper and facilitated the cutting of the glucose-containing sections.

Phenol, even in minute amounts, prevents the estimation of reducing sugars by the Hagedorn-Jensen method, because on addition of the zinc sulphate a brown colour develops and the end-point may last over the addition of 0.5 ml. thiosulphate. The

'blank' values in such instances were raised to 1.50 ml. thiosulphate. When for any reason phenol had been used, all the apparatus and the drying oven were washed with ethanol before further quantitative work was carried out.

Fermentation method

The method used was similar to that described by Mann (1946), with the following slight changes in procedure. The yeast (fresh baker's yeast) was twice washed in the centrifuge with 0.1 M-phosphate buffer (pH 6.8) and resuspended in buffer so that 2 ml. suspension contained 0.7 g. yeast. This volume was added to 5 ml. of a suitably diluted sample of neutralized hydrolysate and the whole incubated at 30° for 1 hr. After centrifuging, a portion of the supernatant liquid was removed for estimation of reducing substances by the Hagedorn-Jensen method. The difference between this value and the value obtained for the unfermented hydrolysate was termed 'fermentable reducing substances'.

Qualitative analysis of sugars in hydrolysates of rumen micro-organisms

In all these experiments acetone powders of the rumen micro-organisms were hydrolysed as already described, and the hydrolysates were passed first over 'Zeo-Karb 215' (Permutit Co. London) and then over 'Deacidite β' (Permutit Co. London) (Partridge, 1948). The solutions were concentrated and subjected to paper chromatography in phenol, collidine, and the mixture of *n*-butanol, acetic acid and water. Glucose was shown to be present as the major component together with some arabinose, xylose, ribose and rhamnose. The ribose presumably arose from nucleic acid. The other pentoses were probably derived from small plant particles which could not be easily removed in the preparation of the acetone powders. No other sugars were detected.

Table 1. Effect of casein on recovery of glucose after hydrolysis with N-HCl

Substance tested	Casein (mg.)	Glucose			
		Before hydrolysis (mg.)	After hydrolysis (mg.)	Corrected for casein (mg.)	Loss (mg.)
Glucose with casein	145.3	24.0	29.0	22.0	2.0
Glucose with casein	127.0	24.0	28.4	21.9	2.1
Casein	139.9	0	7.0	—	—
Casein	165.3	—	7.7	—	—
Glucose	—	24.0	23.3	—	0.7
Glucose	—	24.0	23.6	—	0.4

The production of reducing substances from the acetone powders appeared to be greatest after hydrolysis for 3 hr. Thus in one experiment the production amounted to 10.6 % after 3 hr. as compared with 9.2 % after both 1 and 2 hr. and 10.0 % after 4 hr. The results of tests in which glucose was subjected to the hydrolysis treatment for 3 or 4 hr. alone and also in the presence of casein are shown Table 1, and results for the recovery of glucose added to the acetone powders before hydrolysis are given in Table 2. Glucose recovery in the acetone powders appeared to be similar to that

Table 2. *Recovery of glucose after hydrolysis in the presence of acetone powders of micro-organisms*

Acetone powder (mg.)	Added (mg.)	Glucose			Loss (mg.)
		Recovered after hydrolysing for			
		3 hr. (mg.)	4 hr. (mg.)		
89.0	4.4	4.2	—	0.2	
89.0	4.4	4.1	—	0.3	
89.0	4.4	4.3	—	0.1	
—	4.4	4.3	—	0.1	
45.0	17.1	—	15.3	1.8	
68.5	17.1	—	16.0	1.1	
—	17.1	—	16.4	0.7	

obtained in the presence of casein. It would seem, therefore, that the loss of glucose was likely to be due mainly to the presence of protein in the micro-organisms.

Attempted chromatography of the hydrolysate. Early attempts to obtain chromatograms were made by concentrating the supernatant liquids from the centrifuged hydrolysates in vacuo over phosphorus pentoxide and potassium hydroxide. The dark brown residues amounted to 60 % of the dry weight of the powders, and chromatograms obtained from solutions of these showed gross contamination. The use of ion exchange resins 'Zeo-Karb 215' and 'Deacidite β ', though leading to well-defined chromatograms, presented considerable technical difficulty for routine work and so was abandoned. In an attempt to overcome this difficulty various methods were examined. These consisted of (a) the possible extraction of the glucose-containing substances prior to hydrolysis, and (b) other methods of purification of the hydrolysates.

Extraction experiments. Since it has been postulated that the major glucose-containing substances in rumen micro-organisms resemble starch (Baker & Harriss, 1947-8), starch (A. R., British Drug Houses Ltd.) was selected as a reference substance. Schoch & Jensen (1940) have shown that starch solutions buffered between pH 5.9 and 6.3 may be autoclaved for relatively long periods without a change in the 'alkali number', which is said to be a more sensitive criterion of chemical change in the starch molecule than the reducing value as estimated by the copper or ferricyanide reduction-values (Farley & Hixon, 1941; Schoch, 1945). When this treatment was applied to starch solutions buffered to pH 5.9 at room temperature with Sørensen's citrate buffer, in 3 hr. at 100° there was a loss of about 10 % in the reducing value of the starch subsequently hydrolysed and precipitated (see Table 3). A similar treatment of acetone powders showed that not all the reducing substances were extracted (see Table 4).

It was thought that, if the total reducing values determined were due partly to substances other than glucose, the citrate treatment might not be extracting these substances. In this case there would be a difference in reducing values between the hydrolysed extracts and the unextracted sediments. The reducing substances fermentable by yeast in both the hydrolysed extracts and the unextracted sediment were estimated. The results, presented in Table 5, showed that there were still some fermentable reducing substances in the sediment not extracted with citrate buffer.

This approach was therefore abandoned.

Table 3. Loss of starch from solutions buffered with citrate after hydrolysis

Solution	Treatment	First pre- cipitate* (mg.)	Second pre- cipitate† (mg.)	Difference (mg.)	Difference (%)	Glucose after hydrolysis	
						(mg.)	As percentage second pre- cipitate
Starch and buffer	Hydrolysis for 3 hr. at 100° before precipitation	19.6	15.8	-3.7	—	14.2	89.6
		19.4	15.8	-3.5	—	14.2	90.2
Starch	Precipitation with 5 vol. ethanol	16.6	16.0	-0.6	-3.78	15.8	98.8
		16.2	15.5	-0.6	-4.08	15.4	99.5
Starch with N-HCl	Hydrolysis for 3 hr. at 100°	—	—	—	—	16.6	—
		—	—	—	—	16.4	—
		—	—	—	—	16.4	—

* All weights are given as glucose equivalents of the starch weighed, i.e. weight + 10 %.

† The starch was precipitated twice to remove traces of sodium citrate contained in the first precipitate.

Table 4. Extraction of reducing substances from acetone powders of micro-organisms by hydrolysis in citrate buffer

Powder taken (mg.)	Hydrolysis in	Reducing substances extracted after hydrolysis for	
		3 hr. (%)	6 hr. (%)
181.9	N-HCl	12.0	—
199.5		12.3	—
200.9	Citrate buffer	6.6	—
199.3		6.5	—
200.9	N-HCl	—	12.6
205.5		—	12.5
201.0	Citrate buffer	—	7.6
203.0		—	7.1

Table 5. Fermentable, non-fermentable and total reducing substances in citrate extracts of acetone powders of micro-organisms

Powder taken (mg.)	Hydrolysis in	Reducing substances				
		Before fermentation (mg.)	After fermentation (mg.)	Difference (mg.)	Ferment- able (%)	Non- fermentable (%)
206.0	N-HCl for 3 hr. at 100°	26.0	18.6	-7.4	3.6	9.0
208.8		24.9	18.3	-6.6	3.2	8.8
205.3		25.3	18.1	-7.2	3.5	8.8
206.3	Citrate buffer	10.1	7.6	-2.5	1.2	3.7
177.0		10.0	7.2	-2.8	1.6	4.1

Purification of the acid hydrolysates. It was found that if the acid hydrolysates were neutralized to Congo red with silver carbonate, clear, almost colourless supernatant

liquids could be obtained on centrifuging the mixtures. These could be concentrated under reduced pressure, and the residues, when dissolved in a small volume of water and spotted on to paper, gave well-defined chromatograms though they were not completely free from contamination. Glucose was recovered from acid solution after neutralization with silver carbonate and chromatography to the extent of 94 % with a range from 87 to 99 % (see Table 6), and the reference starch similarly treated after

Table 6. *Recovery of glucose by paper chromatography from acid solution after treatment with silver carbonate*

Solution taken (μ l.)	Glucose		Loss	
	Taken (μ g.)	Recovered (μ g.)	μ g.	%
35.5	236	222	14	5.9
47.0	312	272	40	12.8
42.5	282	259	23	8.1
28.5	190	184	6	3.2
33.5	223	220	3	1.3
44.0	292	271	21	7.2
35.5	236	231	5	2.1
40.0	266	246	20	7.7

hydrolysis was recovered to a somewhat similar degree (see Table 10). Although these values appear to vary appreciably when calculated as a percentage of the amount added to the chromatogram, it will be seen from Table 7 that the variation in the actual content of material was relatively small. Since the percentage error would increase as the quantity of glucose added was decreased, quantities of 250–300 μ g. glucose were added to the paper.

Table 7. *Recovery of starch (as glucose) after hydrolysis and paper chromatography*

Taken (mg./ml.)	Recovered (mg./ml.)	Loss	
		mg./ml.	%
5.04	4.56	0.48	9.5
5.05	4.40	0.65	12.8
5.01	4.80	0.21	4.2

Experiments on the fermentable fraction. As noted on p. 79, the hydrolysates contained a large percentage of a non-fermentable reducing substance. It was thought that this might be produced during hydrolysis (cf. Sattler, 1948). An estimation of the fermentable reducing substances produced after hydrolysis for 1 and 3 hr. (Table 8) showed that this was not likely to be so. The decrease in the percentage of fermentable reducing substances between 1 and 3 hr. hydrolysis was small but definite, about 0.25 mg. glucose, and was of the same order as the loss of glucose shown in Table 2. The increase in the percentage of non-fermentable reducing substances is considered to have been due to a further hydrolysis of those substances that gave rise to them in the sediment.

In an attempt to obtain a more accurate assessment of the fermentable fraction,

Table 8. *Fermentable, non-fermentable and total reducing substances in hydrolysates of acetone powders of rumen micro-organisms*

Powder taken (mg.)	Hydrolysis in N-HCl for	Glucose			Reducing substances		
		Before fermentation (mg.)	After fermentation (mg.)	Difference (mg.)	Fermentable (%)	Non-fermentable (%)	Total (%)
204.7	1 hr.	25.3	15.7	−9.6	4.70	7.65	12.35
204.3	1 hr.	25.3	15.8	−9.5	4.70	7.65	12.35
204.0	3 hr.	26.8	17.7	−9.1	4.45	8.67	13.12
208.3	3 hr.	26.5	17.4	−9.1	4.37	8.32	12.69

estimations of reducing substances were carried out with both the Somogyi (1945) micro-sugar reagent and the Hagedorn-Jensen method. A heating period of 20 min. was adopted for the Somogyi reagent to allow for the estimation of any xylose that might be present. The results are presented in Table 9 and showed that the Somogyi method gave a higher result for fermentable reducing substances and a lower result for the non-fermentable substances than the Hagedorn-Jensen method.

Table 9. *Fermentable and non-fermentable reducing substances in acetone powders of rumen micro-organisms estimated by two methods*

Powder taken (mg.)	Reducing substances					
	Fermentable		Non-fermentable		Total	
	Hagedorn-Jensen (%)	Somogyi (%)	Hagedorn-Jensen (%)	Somogyi (%)	Hagedorn-Jensen (%)	Somogyi (%)
208.9	12.8	14.4	6.3	3.6	19.1	18.3
204.8	12.6	14.8	5.2	3.1	17.8	17.8
217.2	2.0	4.1	7.7	2.6	9.7	6.7
203.3	2.1	3.9	7.7	3.3	9.8	7.2
204.2	1.5	3.3	8.0	3.2	9.5	6.5

Chromatograms were obtained from hydrolysates both before and after fermentation. The solutions were passed through 'Zeo-Karb 215' and 'Deacidite β' and the effluents evaporated and extracted with 95 % ethanol. After removal of the ethanol the residues were dissolved in 0.1 ml. water, spotted on to paper and developed in the *n*-butanol-acetic acid-water mixture for 48 hr. Glucose was included as a reference sugar. On drying and spraying with silver nitrate (Partridge, 1948), it was seen that glucose had been removed during fermentation, but xylose, arabinose, ribose, and rhamnose appeared to be unaffected.

A comparison of the results for fermentable reducing substances, as estimated with the Hagedorn-Jensen method, with those for glucose as estimated by the chromatographic method is presented in Table 10. The samples of sediments used were prepared from rumen contents of sheep collected at different times after feeding. The close agreement between these values in Table 10, taken in conjunction with the results in Table 9, suggests that the results given by the Hagedorn-Jensen method for glucose are more accurate than those given by the Somogyi method.

Table 10. *Comparison of glucose determined chromatographically with fermentable reducing substance determined by the Hagedorn-Jensen method in three different samples of acetone powders of rumen micro-organisms*

Sample no.	Glucose (%)	Fermentable reducing substance (%)
1	5.51	6.61
2	4.64	4.12
3	3.58	3.15

Each estimation in the second column is an average for four chromatograms. The values in the third column are averages of duplicates.

DISCUSSION

The first important point to observe is that no explanation can be offered for the apparent loss of starch when this is heated with citrate buffer.

A second point concerns the discrepancy between the values obtained when the percentage of fermentable and non-fermentable reducing sugars was estimated by both the Hagedorn-Jensen and Somogyi methods.

It has long been realized that in the presence of a large amount of protein the estimation of a polysaccharide presents considerable difficulties. These have been discussed by Dagley & Dawes (1949) who hydrolysed a strain of *Escherichia coli* (*Bacterium coli*) with sulphuric acid and estimated by the Hagedorn-Jensen and Somogyi methods the reducing substances formed. They compared the results thus obtained with those obtained by applying the Sahyun (1931) method for glycogen to the bacteria (cf. Dawson & Happold, 1943). The Hagedorn-Jensen method gave a high result and the Sahyun method a low result as compared with the Somogyi method. Dagley & Dawes (1949) assume that the last method gives the more correct result. The experiments reported in this paper (see Tables 8 and 9) would suggest that, with some bacteria, even the Somogyi method can give high results. It seems clear that, unless the carbohydrate can be unequivocally identified and separated, the usual procedures cannot fail to give confusing results. It is suggested that since the chromatographic procedure separates the required carbohydrate, in this case glucose, from the others present and from non-sugar reducing substances, this condition is fulfilled. In such a case one of the several methods available for the micro-estimation of carbohydrates can be used.

The fraction designated 'non-fermentable reducing substances' presents a problem that has not yet been solved. It does not seem reasonable to think that these substances might consist solely of carbohydrates, since only small quantities of the other sugars described could be detected on the chromatogram. In addition it was found (Heald, unpublished observations) that in fractions of micro-organisms free from plant material, the percentage of non-fermentable reducing substances was still 8-9 %. It is suggested that though these substances arise during the hydrolysis procedure they are not artifacts arising by reason of the method of estimation employed. In addition it is suggested that the fraction is a composite fraction arising from several substances. The nature of these substances remains to be determined.

SUMMARY

1. A paper-partition chromatographic method for the estimation of glucose in hydrolysates of rumen micro-organisms has been developed. The micro-organisms are hydrolysed in hydrochloric acid, the chloride-free solutions are concentrated and the glucose is separated from other substances by paper chromatography. Glucose is estimated by the Hagedorn-Jensen method. Glucose recovery by this method averaged 94 % and ranged from 87 to 99 %.

2. Non-fermentable reducing substances were apparently present in large quantities in the hydrolysates of rumen micro-organisms.

3. It was not possible to extract the glucose-containing material from the micro-organisms by means of a citrate buffer.

4. In addition to glucose, the preparations of micro-organisms contained arabinose, xylose, ribose and rhamnose in small quantities.

The author wishes to thank Dr J. Tosic for suggesting the problem and for his guidance and encouragement during the course of the work.

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The Assessment of Glucose-Containing Substances in Rumen Micro-Organisms during a Digestion Cycle in Sheep

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It has been established that the digestion of carbohydrates in the ruminant occurs mainly through the agency of the micro-organisms of the alimentary tract. The evidence for this subject has been reviewed by Elsdén & Phillipson (1948) and Baker & Harriss (1947-8). Most of this digestion occurs in the rumen. Of the cellulose of the diet about 80 % of the total digested disappears in the rumen, the remainder being digested largely in the caecum (Hale, Duncan & Huffman, 1940, 1947; Gray, 1947).

The nature of the products formed from carbohydrates by microbial attack has been the subject of considerable study and it has been shown by Phillipson (1947-8) and Elsdén & Phillipson (1948) that the major portion of these products consists of volatile fatty acids which are absorbed through the rumen wall. It has, however, been suggested by Baker (1942*a, b*, 1946) that the substances synthesized by the micro-organisms, such as microbial carbohydrate and protein, as well as the end-products such as the acids formed by fermentation, are also of considerable importance to the host animal.

It appears to be established (see Hungate, 1950) that the breakdown of cellulose by micro-organisms probably involves the following path:



It seems reasonable to suppose that both fermentation and assimilation of cellobiose and glucose can occur simultaneously and, as Elsdén (1945) has stated, the problem consists of determining the relative importance of these two processes. Until now no quantitative experiments have been reported concerning the carbohydrate content of the rumen micro-organisms during a digestion cycle in the sheep. This digestion cycle has been described by Tosic (1950).

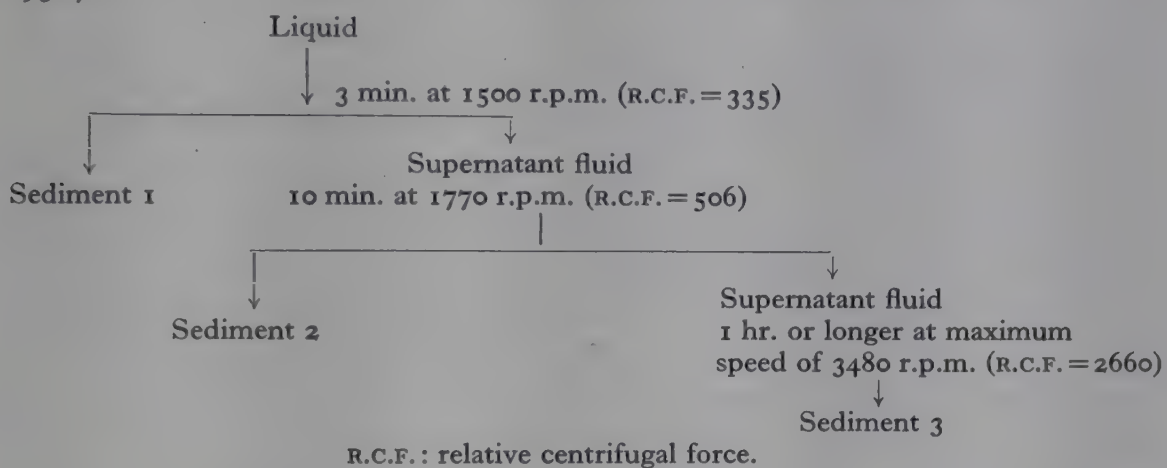
It was shown by Baker & Harriss (1947-8) that not all the micro-organisms of the rumen stain to the same intensity with iodine, and the degree of staining was taken to be a rough indication of polysaccharide synthesis. Since it might be expected that some groups of micro-organisms would have a higher carbohydrate storage than others, the carbohydrate storage of three fractions of rumen micro-organisms from sheep fed on hay was investigated during a 12 hr. digestion cycle. In addition, the quantity of microbial carbohydrate passing out of the abomasum was estimated. This was made possible by using a permanent cannula inserted immediately caudal to the pylorus (Phillipson, 1948).

METHODS

Rumen samples and their fractionation

The rumen samples were collected through an ebonite cannula (Quin, Van der Wath & Myburgh, 1938; Phillipson & Innes, 1939) by means of a wide-bore glass tube. The rumen was massaged before sampling, in order to obtain a more representative sample of the contents (Tosic, 1950).

The samples were collected at intervals of 2, 5, 8 and 12 hr. after a meal of hay, and were strained through S-14 bolting silk (Henry Simon Ltd., Cheadle Heath, Stockport, Cheshire). The strained sample was centrifuged according to the following scheme (Tosic, 1950):



In most of the experiments reported below, sediments 2 and 3 were obtained as one fraction.

The sediments were washed with distilled water, and known volumes were hydrolysed with N-HCl for 3 hr. at 100° in sealed tubes. The weight of sediment hydrolysed was obtained by drying known volumes to constant weight at 105°.

Determination of microbial carbohydrate in the rumen and of that leaving the abomasum

Sheep. Two Suffolk Cross ewes were fitted with rumen cannulas and with cannulas inserted into the duodenum immediately caudal to the pylorus, since it is at this point that the most representative sample of digesta leaving the abomasum is obtained (Phillipson, personal communication). A third animal was fitted only with the abomasal cannula.* For comparison of the samples obtained through these cannulas, four Cheviot wethers of the same age received the same diet and were slaughtered at 2, 5, 8 and 12 hr. after feeding. The sheep received feeds of 750 g. chopped hay at intervals of 12 hr. They usually consumed most of the food within 2 hr., and in the first experiments no attempt was made to confine the food and water intake to a 2 hr. period. In later experiments the sheep were trained to consume within 2 hr. the food and as much water as they required. This avoided any change in the rumen micro-organisms during the next 10 hr. due to a fresh influx of food or water. Weights of food and water consumed were recorded.

* The author is indebted to Dr A. T. Phillipson for placing this animal at his disposal.

Samples. The rumen samples were fractionated as already described. Samples from the duodenal cannulas were strained through bolting silk and as much solid material as possible was removed by centrifugation. The sediment was washed with distilled water and again centrifuged at 6000 r.p.m. The resulting sticky sediment was resuspended in water, and known volumes were hydrolysed by the procedure already described. The dry weights of the sediments were obtained by drying known volumes of the suspensions at 105° to constant weight.

In slaughtered animals the rumen and reticulum, the omasum and abomasum were tied off and removed. The contents of the rumen and reticulum were pooled, and samples of the mixed contents were fractionated in the same way as the samples obtained from live animals through a cannula. The contents of the abomasum were removed and sampled, and the samples treated in the same way as those from the duodenal cannula. Total dry weights were obtained on all samples taken. This permitted a check of the constancy of the samples obtained through the cannulas.

Quantitative estimation of carbohydrates. Glucose and 'fermentable reducing substances' in the hydrolysates were estimated by the method described by Heald (1951).

Estimation of reducing substances fermentable by yeast in abomasal fluid. The samples were collected over periods of 12 hr. after feeding, and were strained through bolting silk. The liquid was centrifuged for 1 hr. at 6000 r.p.m. in an angle centrifuge when, in most instances, a clear supernatant liquid was obtained. This was adjusted with sodium hydroxide to pH 6.0–7.0 (Universal Indicator, British Drug Houses Ltd.), and the reducing substances fermentable by yeast were estimated. A preliminary experiment showed that deproteinization of the liquid (Fujita & Iwatake, 1931) was not necessary (Table 1).

Analysis of hay. Dry matter was determined by drying to constant weight at 105°, the ash content by igniting at 500°, cellulose by the method of Crampton & Maynard (1938), nitrogen by the method of Chibnall, Rees & Williams (1943), water-soluble material by extraction with water at 30°. In this last process the residue was washed on a filter until the washings were clear and the total extract evaporated to dryness before it was weighed. Reducing sugars fermentable by yeast and non-fermentable reducing substances were determined in an ethanolic extract of the hay, prepared according to de Mann & de Heus (1949).

Preparation of α -cellulose. α -Cellulose was prepared from a sample of birch-wood pulp, according to the method described by Dorée (1947).

Hydrolysis of cellulose. A sample of birch-wood pulp* of a high α -cellulose content and a sample of an α -cellulose preparation (Dorée, 1947) were washed with warm distilled water and dried. After hydrolysis, the total reducing substances formed were estimated. From the results presented in Table 2 it was concluded that a maximum of 7 % of the cellulose present would be liberated as glucose, assuming that the reducing substance estimated was glucose.

Hydrolysis of hay. A sample of hay was extracted three times with distilled water at 30° for periods of 1 hr. and washed thoroughly on a filter. It was dried overnight at

* Obtained through the courtesy of Dr Rance and Mr Wilson of Messrs Alex. Pirie Ltd., Stoneywood Mills, Bucksburn, Aberdeenshire.

Table 1. *Effect of removal of protein from abomasal liquids of a sheep before estimation of glucose*

Solution	(Values expressed in mg./100 ml.)					
	Protein removed			Protein not removed		
	Amount of glucose		Difference	Amount of glucose		Difference
	Before fermentation (mg.)	After fermentation (mg.)		Before fermentation (mg.)	After fermentation (mg.)	
Supernatant liquid	19.2	19.3	+0.1	41.8	38.7	-3.1
Supernatant liquid with glucose	21.8	19.3	-2.5	44.4	42.3	-2.1
Glucose	2.6	0.2	-2.4	2.6	0	-2.6

Table 2. *Amount of reducing substances (calculated as glucose) formed on hydrolysis of cellulose under conditions similar to those used for hydrolysis of the sediments from rumen micro-organisms*

Wt. of sample (mg.)	Treatment	Amount of glucose formed from			
		Birch pulp		α-Cellulose	
		(mg.)	(%)	(mg.)	(%)
100.0	Hydrolysed with N-HCl for 3 hr. at 100°	4.5	4.5	—	—
121.9		5.3	4.4	—	—
123.2		5.1	4.2	—	—
303.8		—	—	19.5	6.4
249.7	Heated with water for 3 hr. at 100°	—	—	17.8	7.1
239.1		—	—	15.8	6.6
280.2		—	—	0	0
121.0		0.1	0.1	—	—

105°, and weighed quantities were hydrolysed with N-HCl. Since it was expected that pentoses would be present in the hydrolysate, the reducing substances fermentable by yeast were estimated. The results are given in Table 3.

Table 3. *Amount of fermentable, non-fermentable, and total reducing substances in a hay hydrolysate*

Hay (mg.)	Treatment	Reducing substances		
		Fermentable (%)	Non-fermentable (%)	Total (%)
239.1	Hydrolysed with N-HCl for 3 hr. at 100°	4.2	42.0	46.2
280.2		5.0	45.4	50.4
168.2	Heated with water for 3 hr. at 100°	0	3.9	3.9

In order to obtain some information concerning the nature of the non-fermentable reducing substances, samples of the hydrolysates were neutralized with silver carbonate. The clear supernatant fluids obtained on centrifuging were spotted on to Whatman no. 1 paper and the chromatograms were developed with a mixture of *n*-butanol, acetic acid and water, and also with phenol (Partridge, 1948) and with a mixture of pyridine-*n*-amyl alcohol and water (Werner & Odin, 1949) using glucose, arabinose and xylose as reference sugars.

A faint glucose spot and an intense xylose spot were detected when the dried chromatograms were sprayed with ammoniacal silver nitrate (Partridge, 1948).

Qualitative analysis of the water-soluble sugars in the hay. Hay (20 g.) was extracted four times at 37° for periods of 30 min. with 100 ml. water. The combined extracts were evaporated to a small volume (20 ml.) at 40° under reduced pressure, and 4 vol. 95 % (v/v) ethanol were added. The precipitate obtained was centrifuged and the supernatant liquid was removed and evaporated to dryness at 40° under reduced pressure. The dry residue was dissolved in 0.5 ml. distilled water and the solution was spotted on to Whatman no. 1 paper together with a solution of glucose, sucrose and fructose as reference sugars. The chromatograms were developed for 72 hr. in *n*-butanol water, dried at 105° and sprayed with naphthoresorcinol (Forsyth, 1948) to detect fructose and sucrose, and with aniline hydrogen phthalate (Partridge, 1949) to detect glucose. An intense fructose spot and a faint glucose spot were observed.

The precipitated material (50 mg.) was hydrolysed in a sealed tube with 0.1 N-H₂SO₄ (1 ml.) for 1 hr. at 100°. Not all the precipitate dissolved. The hydrolysate was adjusted to pH 4.5 (Congo red) by addition of barium carbonate and centrifuged. The clear supernatant fluids were spotted on to paper and the chromatograms developed with *n*-butanol water for 72 hr. Glucose and fructose were included as reference sugars. The chromatograms were dried at 105° and sprayed with naphthoresorcinol and aniline hydrogen phthalate. Fructose was the main carbohydrate detected, but there were also some red streaks leading downwards from the point of application of the hydrolysate. These might have been fructose polymers incompletely hydrolysed.

RESULTS

Glucose contents of sediment 1 and of sediments 2 and 3 together. The results of two determinations of glucose and of fermentable and non-fermentable reducing substances on sediment 1 and sediments 2 and 3 are shown in Fig. 1. Essentially similar graphs were obtained by analysis of sediments from two other sheep. The contents of glucose and of fermentable reducing substances were in close agreement at all stages of the digestion cycle. The content of non-fermentable reducing substances varied a little throughout the cycle and was the same for both sediments.

The content of glucose in hydrolysates from sediment 1 was consistently higher than that of glucose in the hydrolysates from sediments 2 and 3. It was thought that this difference might be due to glucose produced by the hydrolysis of cellulose or similar materials contained in the sediment as plant particles, since it is known that plant particles were present (Tosic, 1950). Experiments were therefore carried out to determine the cellulose content of the sediment and the results (Table 4) were compared with those of hydrolysis of cellulose (Table 2) and of hay (Table 3) already reported.

Cellulose in sediment 1. Cellulose was estimated in an acetone powder (see Heald, 1951) of sediment 1, but no correction was made for the ash content. From the results in Tables 2 and 4, it was calculated that the maximum quantity of glucose that might be produced from hydrolysis of the cellulose residues in sediment 1 was 0.5–0.6 %. The glucose values shown for sediment 1 in Fig. 1 would, therefore, appear to be too high by this amount.

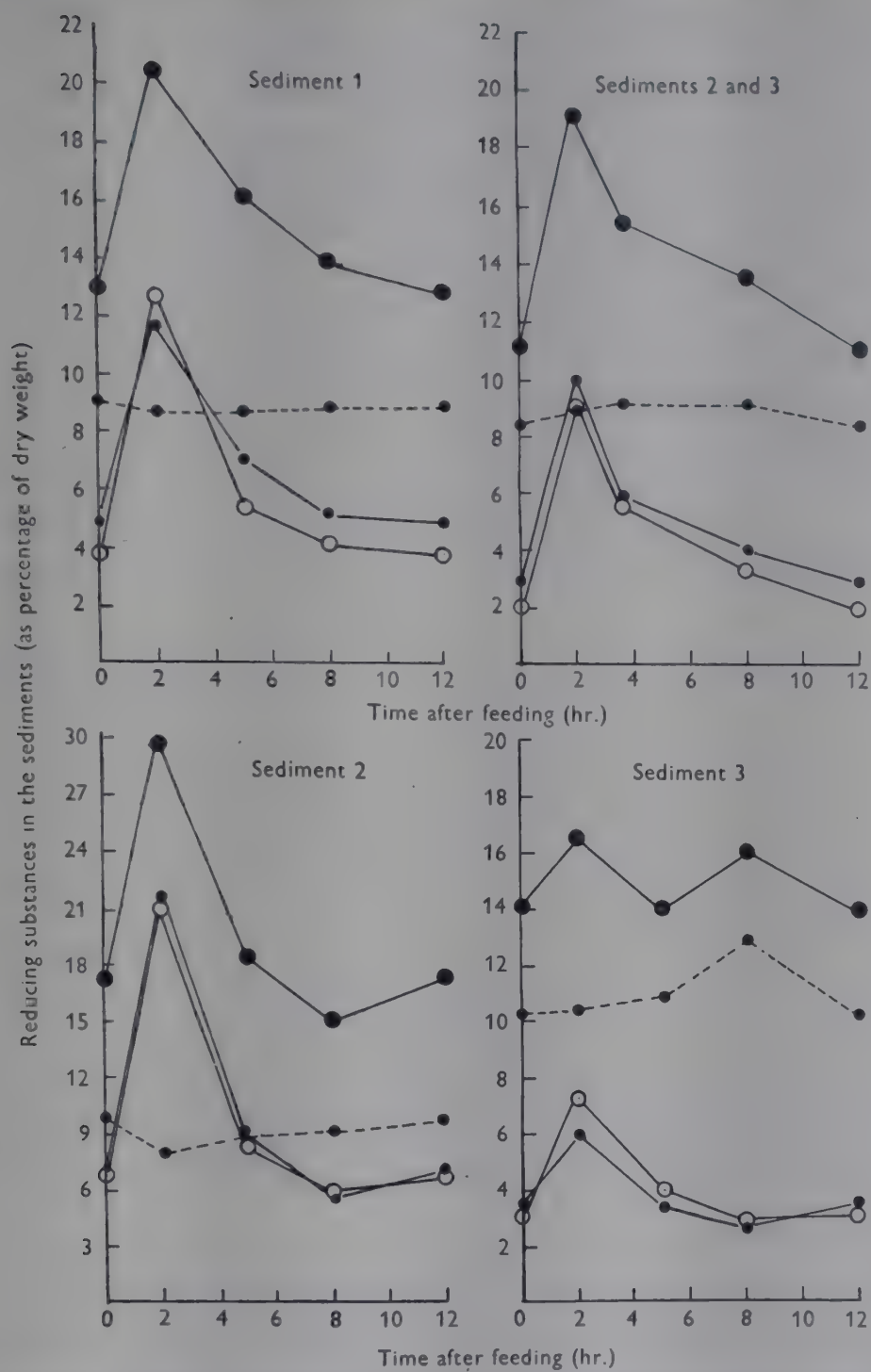


Fig. 1. Changes in the quantities of reducing substances in the sediments centrifuged from rumen liquor. Results are shown for sediments 1, and 2 and 3 together, for one sheep and for sediments 2 and 3 separately, for another sheep. ●—●, total reducing substances; ○—○, glucose-containing carbohydrate; ●—●, fermentable reducing substances; ●— — ●, non-fermentable reducing substances.

Table 4. Cellulose content of acetone powder of sediment 1 from rumen micro-organisms

Exp. no.	Sediment taken (mg.)	Cellulose residue*	
		mg.	As percentage of sediment
1	225.5	17.9	7.9
2	273.0	24.5	8.9
3	339.6	29.3	8.6

* Not corrected for ash content.

Glucose contents of sediments 1, 2, 3 and the cellulose content of sediment 1 determined concurrently. To correlate the previous results the contents of glucose were estimated in the three sediments and the content of cellulose in sediment 1 from one sheep. The feed for the sheep consisted of 1180 g. chopped hay given in two portions of 590 g. at 12 hr. intervals. The hay contained (on a dry-weight basis) 14.3 % water-soluble matter, 0.45 % total reducing substances calculated as glucose, and 0.33 % yeast-fermentable reducing substances calculated as glucose (Tosic, personal communication). The animal consumed all the food within 2 hr. The results for sediment 1 in this experiment were essentially the same as those obtained for sediment 1 in a previous experiment and recorded in Fig. 1. The results for sediments 2 and 3 in the present experiment are also shown in Fig. 1.

From the dry weights of the sediments obtained from the rumen samples it was calculated that the dry weights of the rumen fractions in mg./100 g. wet rumen sample were of the following order: sediment 1, 500–600; sediment 2, 60–100; sediment 3, 250–300.

It was further assumed that the rate of passage of material out of the rumen into the abomasum was of the same order as that of material passing out of the abomasum into the duodenum, i.e. 400 ml./hr. This figure is probably high (Phillipson, Green, Reid & Vowles, 1949), but calculated in this way, the quantity of glucose-containing substances in the micro-organisms passing out of the rumen in 24 hr. was of the order of 5 g.

Glucose liberation in the abomasum. Consideration of the above calculation suggested at least three criticisms that could be applied to it and might account for the low value for glucose-containing substances. First, no assessment could be made of the glucose-containing substances in the micro-organisms adhering to the plant particles (Baker & Harriss, 1947–8), removed on straining the rumen liquor. Secondly, the relatively long centrifuging procedure results in a rise in temperature and could have led to autolysis of the fractions. Thirdly, since the samples were obtained through a cannula, calculations based on dry-matter content of the liquid sample cannot be directly applied to the actual rumen contents.

In order to overcome the second and third objections, an attempt was made to estimate the glucose-containing substances in the sediment obtained from the abomasum, and in the partly digested food leaving the abomasum. To get a comparison with the results obtained with samples removed through the cannulas, sheep were slaughtered at intervals after feeding and samples of the rumen and abomasal contents were prepared as described on p. 86.

It has been suggested (cf. Baker, 1946) that in the abomasum the micro-organisms entering from the rumen are broken down. Since this may also involve a breakdown of microbial carbohydrate, an estimation of the carbohydrate alone in any sediment of micro-organisms obtained would give a wrong value for the quantity of such carbohydrate passing through to the rest of the alimentary tract. The results of estimating reducing substances fermentable by yeast in the liquid leaving the abomasum are presented in Table 5, and show that scarcely any fermentable reducing substances were liberated in the abomasum. The carbohydrate content of micro-organisms leaving the abomasum and of those in the rumen were then estimated as already described.

Table 5. *Fermentable reducing substances in abomasal liquid of a sheep*
(Values expressed in mg./100 ml.)

Exp. no.	Amount formed			
	12 hr. after feeding	2 hr. after feeding	5 hr. after feeding	8 hr. after feeding
1	1.5	1.6	1.2	2.2
2	2.5	4.1	1.8	6.5

Glucose content of the microbial sediment leaving the abomasum. The results obtained from the two cannulated animals and from the slaughtered animals are presented in Fig. 2. Only the content of glucose is shown, though in all instances those of yeast-fermentable and non-fermentable reducing substances were estimated and were found to correspond to the values shown in Fig. 1.

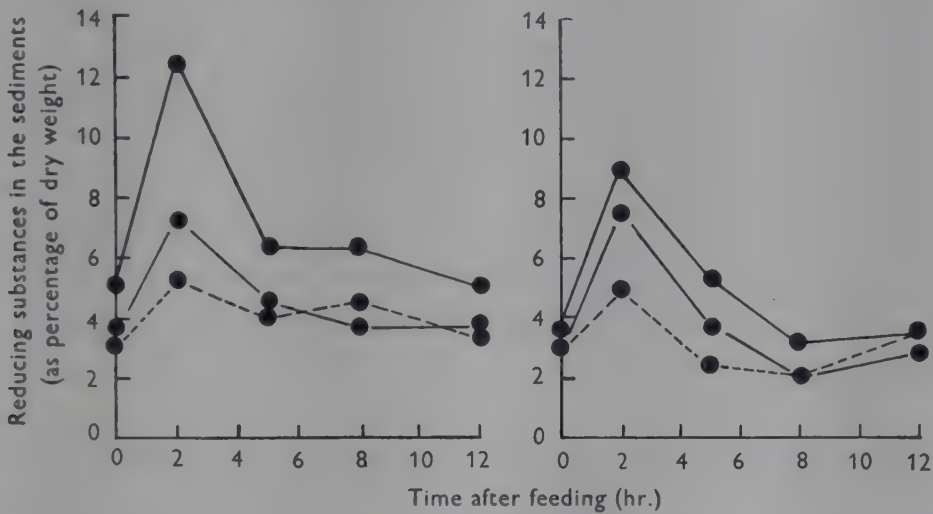


Fig. 2. Changes in the quantities of reducing substances in the sediment centrifuged from rumen and abomasal liquor. Results are shown for samples taken through rumen and duodenal cannulas, and for samples taken from slaughtered sheep. ●—●, carbohydrate in sediment 1; ●—●, carbohydrate in sediments (2 and 3); ●---●, carbohydrate in the sediment from the abomasum.

The average dry weight of the total sediment centrifuged from the strained abomasal samples was 0.8–1.0 % of the wet sample. By using this figure, by taking the average amount of glucose-containing material in this sediment as 5 %, and by assuming that the rate of passage of food through the abomasum is 400 ml./hr. (Phillipson *et al.* 1949), it was calculated that the quantity of glucose in combined form in the micro-organisms passing into the duodenum was probably of the order of 5 g./24 hr.

DISCUSSION

This paper deals with two aspects of carbohydrate synthesis and storage by the rumen micro-organisms. The most definite fact arising is that for sheep fed on hay the quantity of glucose-containing carbohydrate stored in the micro-organisms and passing into the duodenum is nutritionally insignificant. On a hay diet, the main nutritional value of the carbohydrate broken down by the micro-organisms of the rumen lies in its subsequent fermentation to fatty acids (cf. Marston, 1948). That this fermentation may be preceded by a storage of carbohydrate within the micro-organisms in the rumen

does not affect this conclusion, since utilization of the stored carbohydrate by the micro-organisms may again result in further production of fatty acids. Work to investigate this problem is in progress.

The second point arising concerns the rapid increase in carbohydrate content of the micro-organisms immediately after a meal, followed by an equally rapid decline. This change is especially pronounced with sediment 2, the percentage of carbohydrate rising from 6.5 to 21–22 in 2 hr. and falling to 9 within 5 hr. after feeding (Fig. 1). A possible explanation of this increase might be that it is due mainly to the influx of soluble carbohydrates in the food, which in the hay used consisted mainly of fructose and fructosans. It is to be noted, however, that on a total quantity basis sediment 1 accounted for the greater amount of carbohydrate stored. These figures suggest that the different groups of micro-organisms of the rumen assimilate the soluble sugars to different degrees, but further experiments are necessary to decide this and to assess the significance, if any, of such storage. The close agreement observed between the yeast-fermentable reducing substances and the glucose content of the sediments suggests that for sheep fed on a hay diet this method, rather than the more prolonged chromatographic procedure, might be used in preliminary experiments.

The almost constant content of non-fermentable reducing substances has been discussed in a previous paper (Heald, 1951). It is of interest that this fraction is present to the same extent in the micro-organisms obtained from the abomasum as in those from the rumen. This would suggest that if such substances are liberated by digestion of the micro-organisms, the process must take place lower down in the alimentary tract.

The amounts of total reducing substances estimated are great and, if taken to indicate glucose, would lead to a wrong assessment of the value of microbial carbohydrate to the animal. For example, the total reducing substances in the abomasal hydrolysates ranged from 13 to 15 % of the dry weight of the micro-organisms. Taking this figure as the glucose content, the calculation applied on p. 91 would lead to a figure of 13–15 g./24 hr., whereas the value is more likely to be 5–6 g. This error is due in part to the use of the Hagedorn-Jensen reagent, but it has been shown (Heald, 1951) that with the Somogyi (1945) reagent the error in estimating the quantity of carbohydrate in hydrolysates of rumen micro-organisms would be greater.

SUMMARY

1. A study has been made of the carbohydrate content of the rumen micro-organisms during a digestion cycle in sheep. Samples of liquid from the rumen and abomasum were obtained from slaughtered animals and also by cannulas from live animals.

2. The average amount of glucose in the micro-organisms from the abomasum of hay-fed sheep was 4–5 % of the dry weight, and the total quantity passing from the abomasum was calculated to be 5–6 g./24 hr. This quantity can be of little importance to the sheep.

3. The storage of glucose-containing carbohydrate within the rumen by three fractions of micro-organisms was studied and was found to undergo a marked increase immediately after a meal. This increase was followed by a rapid fall.

4. It is pointed out that the use of reducing methods for estimation of sugars in hydrolysates of micro-organisms can lead to a false assessment of their carbohydrate content.

The author's thanks are due to Dr J. Tosic for suggesting this problem and for his interest and advice, and to Dr A. T. Phillipson for fitting two sheep with duodenal cannulas, and for helpful discussions.

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PROCEEDINGS OF THE NUTRITION SOCIETY

SIXTY-SECOND SCIENTIFIC MEETING
DERBY HALL, LIVERPOOL

23 SEPTEMBER 1950

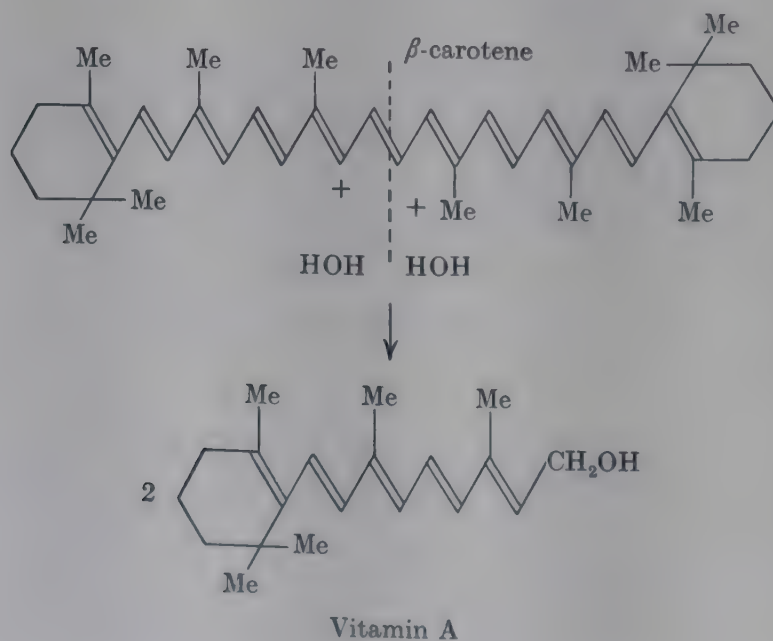
VITAMIN A

Chairmen: MISS E. M. HUME, *Lister Institute of Preventive Medicine, London, S.W. 1.*
DR S. K. KON, *National Institute for Research in Dairying, University of Reading*

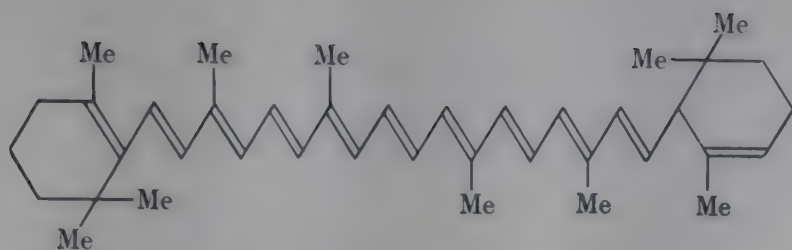
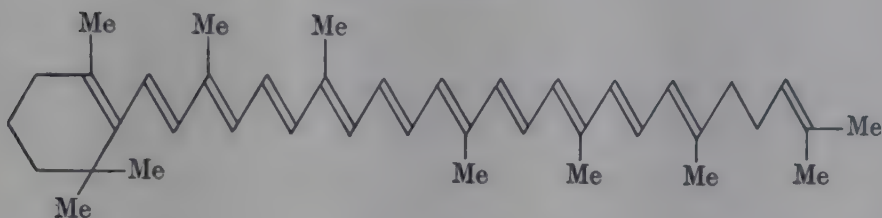
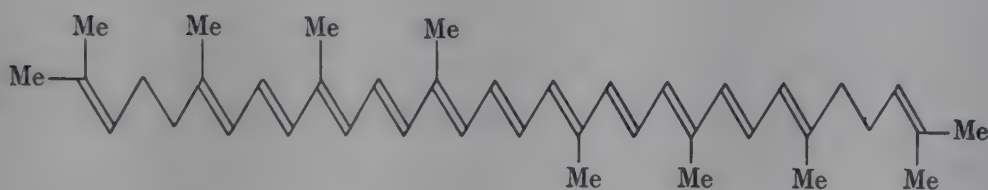
Vitamin A-Active Substances

By T. W. GOODWIN, *Department of Biochemistry, University of Liverpool*

Animals obtain their vitamin A either as such or as certain carotenoids which can be converted into vitamin A; such carotenoids are termed vitamin A precursors or provitamins A. For convenience, carotenoids and vitamin A and its derivatives can here be considered separately, but it should be emphasized that this differentiation is in a sense artificial because carotenoids are the ultimate source of all vitamin A. The carotenoid possessing the greatest vitamin A activity is β -carotene and the conversion can be represented qualitatively as a hydrolytic cleavage thus:

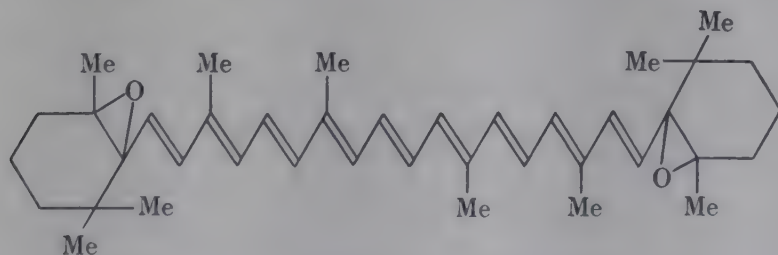


As α - and γ -carotenes are about one-half as active as β -carotene (Underhill & Coward, 1939; Kuhn & Brockmann, 1933; Wilkinson, 1941; von Euler, Karrer, Hellström & Rydbom, 1931) and lycopene is completely inactive (Karrer & Jucker, 1948), it follows that a β -ionone residue is a first essential for activity.

 α -Carotene γ -Carotene

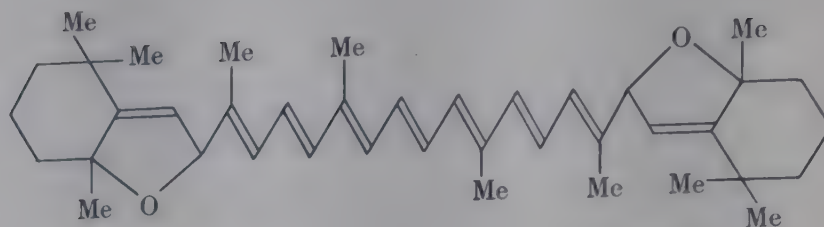
Lycopene

The complete inactivity of zeaxanthin (3:3'-dihydroxy- β -carotene) and of lutein (3:3'-dihydroxy- α -carotene) (von Euler, Karrer & Zubrys, 1934) and the relative activity of cryptoxanthin (3-hydroxy- β -carotene) which is about one-half that of β -carotene* (see Zechmeister, 1949) further indicates that the β -ionone residue must be unsubstituted; a very recent claim that astaxanthin (3:3'-dihydroxy-4:4'-diketo- β -carotene) is active in fish (Grangaud & Massonet, 1950) must, at the moment, be treated with great reservation. Recent work by Karrer and his associates suggests, however, that this criterion may have to be modified. Their recent work on the production of carotenoid epoxides indicates that 5:6-epoxides, e.g. 5:6-5':6'-diepoxy- β -carotene, are vitamin A precursors (Karrer, Jucker, Rutschmann & Steinlin, 1945).

5:6-5':6'-Diepoxy- β -carotene

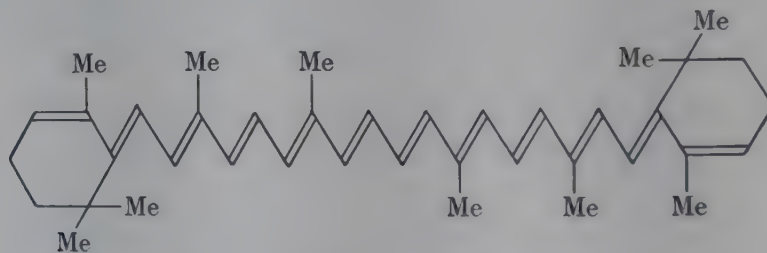
It remains to be seen whether the criterion of an unsubstituted β -ionone residue will need to be discarded *in toto*, because it is quite possible that these epoxides are not active *per se* but by virtue of the fact that they are first converted into β -carotene. The furanoid (5:8-) epoxides, e.g. aurochrome (Karrer *et al.* 1945) are inactive.

* If vitamin A storage instead of growth-promoting power is taken as the criterion of activity, cryptoxanthin appears to be almost as effective as β -carotene (Johnson & Baumann, 1948).

(Aurochrome) 5:8-5':8'-diepoxy- β -carotene

The presence of a furanoid group does not, however, interfere with activity if the other end of the molecule contains an appropriate residue, e.g. mutatochrome (5:8-epoxy- β -carotene) and luteochrome (5:6-5':8'-diepoxy- β -carotene) are active (Karrer & R  gger, 1940; Gridgeman, Hunter & Williams, 1947).

The integrity of the isoprene side chain is a further essential requirement for full vitamin A activity, although it appears possible that some variations can be made without completely destroying activity (see Johnson, 1950). Two examples will clearly illustrate this. Hydrogenation of β -carotene with aluminium amalgam produces β -dihydrocarotene (7:8, 7':8'-tetrahydro- β -carotene); this compound is without vitamin A activity (Karrer & R  gger, 1940) as is the completely hydrogenated perhydro- β -carotene (von Euler, Demole, Karrer & Walker, 1930). Decomposition of the β -carotene- I_2 addition product with, for example, thiosulphate, yields *isocarotene* (dehydro- β -carotene) in which all activity is destroyed because a rearrangement of double bonds occurs, thus:

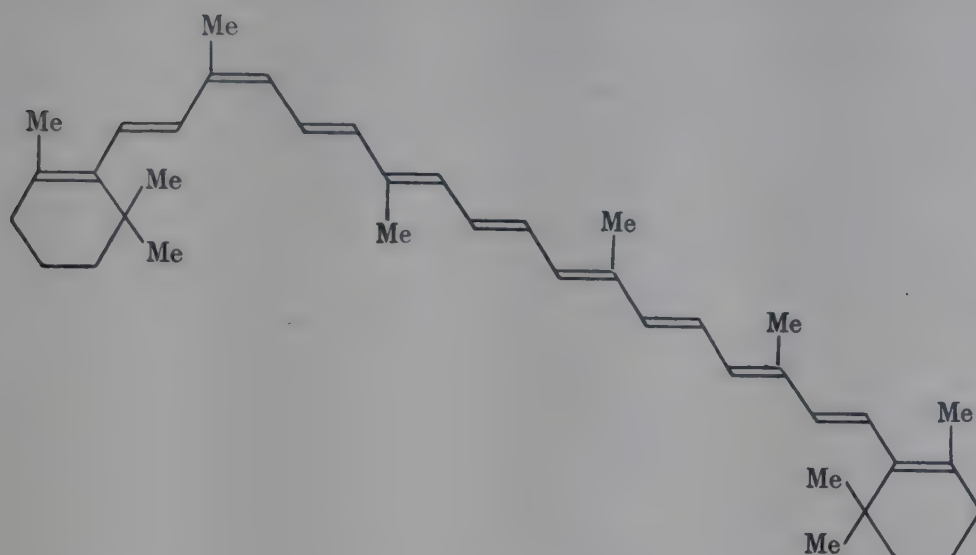
Dehydro- β -carotene

(Karrer & Schwab, 1940).

Unilateral oxidative degradation of the β -carotene molecule with the production of β -apocarotenes does not destroy activity so long as a vitamin A side chain remains present in the molecule, e.g. β -apo-8'-carotenal and β -apo-12'-carotenal are about as active as α -carotene (Karrer & Solmssen, 1937; Karrer, R  gger & Geiger, 1938). α -Apocarotenes, on the other hand, e.g. α -apo-8-carotenal, are inactive because only an α -ionone residue remains (von Euler, Karrer & Solmssen, 1938).

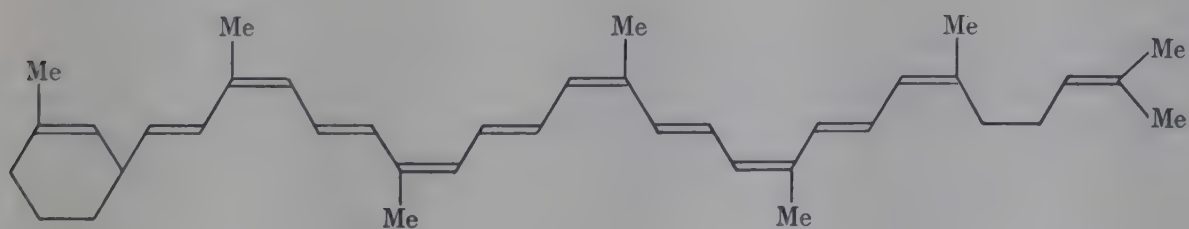
Carotenoids can exist in a number of stereoisomeric forms (see Zechmeister (1944) for a full discussion), but in general the naturally occurring form is the all-*trans* form. Under suitable conditions these all-*trans* forms can easily be converted into *cis*-isomers. Deuel and Zechmeister and their collaborators have, in a long series of papers (see Zechmeister (1949) and Goodwin (1951) for full references) measured the vitamin A activities of many of these *cis*-isomers, and have found that, with one important exception (see below), they are less active than the corresponding all-*trans* compounds. Some of these results have recently been independently confirmed by Bahl, Sadana & Ahmad (1948). A *trans* $\rightarrow$ *cis* rotation results in general in a carotenoid losing its

straight shape, e.g. neo- β -carotene U (*3*^{*}-mono-*cis*- β -carotene), and Deuel and Zechmeister suggest that the lowered activity of *cis* carotenoids is due to the difficulty of the non-linear molecule fitting on to the 'carotenase' enzyme system.



Neo- β -carotene U

There remains the possibility, however, that neo-carotenoids are not active *per se* but are first re-arranged in the intestinal tract to β -carotene; in fact, Kemmerer & Fraps (1945) state that they have obtained evidence of such a re-arrangement. Deuel's and Zechmeister's objection to this suggestion is based on their experience with pro- γ -carotene. This carotenoid is a naturally occurring poly-*cis*-isomer (Zechmeister terms such naturally occurring *cis*-compounds 'pro-carotenoids'), probably 3:5:7:9:11-penta *cis*- γ -carotene, which has a biological activity indistinguishable from that of the all-*trans*- γ -carotene. In pro- γ -carotene all possible *trans* $\rightarrow$ *cis* rotations have occurred, for rotation of all the double bonds cannot take place owing to steric hindrance (Zechmeister, 1944), and the molecule is no longer bent:



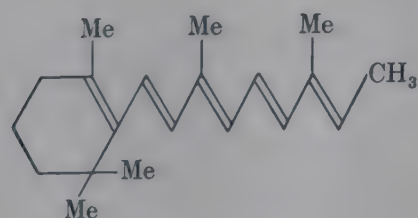
Pro- γ -carotene

This molecule, it is assumed, can fit on to the appropriate enzyme as easily as all-*trans*- γ -carotene and thus the two compounds have the same vitamin A activity.

Vitamin A is a primary alcohol, and it is interesting to observe the effect on the biological activity of the molecule of altering the terminal hydroxyl grouping. Vitamin A esters are of course active, being hydrolysed in the gut, absorbed in the free state and re-esterified during the passage across the gut wall (Gray, Morgareidge & Cawley,

* In Zechmeister's nomenclature italicized numerals refer to the double bonds in the molecule and not to the carbon atoms.

1940). Retinene (vitamin A aldehyde) is active (Glover, Goodwin & Morton, 1948a) and so are the ethers derived from vitamin A and its higher homologues (see Embree (1947) and Milas (1947) for full references), vitamin A acid (Arens & van Dorp, 1946; Sharman, 1949) and dimethylaminovitamin A (Milas, 1947). Karrer & Benz (1948) prepared the hydrocarbon corresponding to vitamin A (axerophthene) and found that it was biologically active; they concluded that the substituents on the terminal carbon



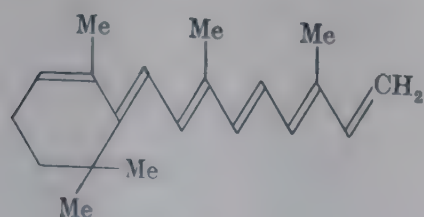
Axerophthene

atoms were of little significance in controlling activity. Taken with the results just quoted this appears to be valid, but it should be noted, however, that Karrer, Patel & Benz (1949) have recently reported that 15-ethylaxerophthene is inactive, and that Meunier and his collaborators (Meunier, 1948; Guerillot-Vinet, Meunier, Jouanneteau & Gourevitch, 1948) deny biological activity to axerophthene. Any shortening of the

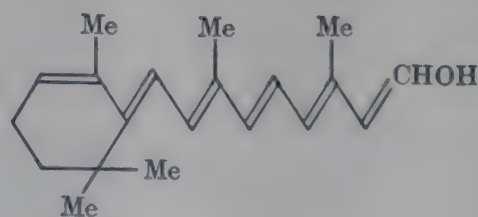
side chain of vitamin A completely destroys biological activity (Karrer & Benz, 1948), but apparently some variations can be made on the side chain without eliminating activity completely. As full details of compounds of this type are not always available, they will not be discussed further. A recent review of the organic chemistry of these compounds which has recently appeared (Johnson, 1950) also included some data on their biological activity.

Vitamin A, according to Zechmeister (1944), can exist in four possible stereoisomeric forms because bonds 3 and 5 are sterically unhindered. Leaving aside the possibility of the compounds not being completely pure, the varying activities reported for synthetic vitamin A derivatives synthesized by different routes may be due to the production of *cis*-isomers (see Milas, 1947; Embree, 1947). Only one vitamin A isomer has as yet been unequivocally identified; Robeson & Baxter (1947) isolated *neo*-vitamin A and consider it to be 5-*cis*-vitamin A. Rather surprisingly, when the activity of *cis*-carotenoids is remembered, it has the same biological potency as vitamin A itself.

The position of anhydrovitamin A is interesting since Shantz (1950) has emphasized that it has a small but significant potency (0.4 % that of vitamin A); as it possesses the same disposition of double bonds as dehydro- β -carotene, this very low value is not



Anhydrovitamin A



Rehydrovitamin A

surprising since the carotenoid is apparently inactive. Anhydrovitamin A is, however, stored in the liver as a compound containing an hydroxyl group; this compound, termed rehydrovitamin A by Shantz, when fed to rats is twenty times as active as anhydrovitamin A. Vitamin A can form a 5:6-epoxide (Karrer & Jucker, 1945) which,

however, appears to have very little activity. Kitol ($C_{40}H_{58}(OH)_2$) which occurs in mammalian liver oils, being especially abundant in whale-liver oils, is apparently a dimer of vitamin A (see Harris, 1949; Barua & Morton, 1949) and has no biological activity *per se*, but yields vitamin A on heat treatment.

A derivative of vitamin A that has recently aroused much interest is vitamin A_2 ; although widely distributed in both marine and freshwater fish this compound is relatively much more abundant in the latter. It is active *per se* in the rat, replacing vitamin A completely without in any way being converted into it (Shantz, Embree, Hodge & Willis, 1946). Its activity is less than that of vitamin A, Shantz & Brinkman (1950) reporting a potency of 1.3×10^6 i.u./g. Evidence is now accumulating that vitamin A_2 is 3-dehydrovitamin A (Morton, Salah & Stubbs, 1947; Morton, Cama, Dalvi, Field & Salah, 1950). If this is so, it means that animals can utilize a dehydro- β -ionone residue; unfortunately no naturally occurring carotenoid is yet known which contains such a residue, and thus a direct demonstration of a conversion of such a compound into vitamin A_2 is not, at the moment, possible. On the other hand, freshwater fish apparently have the ability to dehydrogenate β -carotene in this way, since Morton & Creed (1939) have demonstrated the conversion of β -carotene into vitamin A_2 in dace and perch.

One of the most important recent observations in the study of vitamin A-active substances is undoubtedly that vitamin A acid is potent without apparently being converted into vitamin A (Arens & van Dorp, 1946; Glover, Goodwin & Morton, 1948*b*; Sharman, 1949).

Apart from its function in vision, the major physiological activity of vitamin A is in preserving the integrity of the epithelial tissues but, in spite of this, the presence of vitamin A has never been unequivocally demonstrated at this site of action. The observations on vitamin A acid strongly suggest that we may have been premature in accepting the suggestion that the alcohol is the active form of the vitamin; this may well be only an intermediate in the conversion of the stored vitamin esters into the, as yet unrecognized, active principle.

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Vitamins A and Vision

By R. A. MORTON, *Department of Biochemistry, University of Liverpool*

According to the ideas now current, the following sequence obtains in the formation of visual purple: (1) The animal ingests carotenoid provitamins, and sometimes pre-formed vitamin A, but absorption of unchanged carotenoids is small or very small indeed, depending on the species. (2) An enzyme system (carotenase?) operating in the gut wall results in fission of the C_{40} molecules to C_{20} molecules and vitamin A aldehyde is formed, to be rapidly reduced to free vitamin A. (3) Much of the vitamin A is esterified in the gut wall and then transported to the liver to be stored largely as ester in the Kupffer cells. Either in the true liver cells, or in the blood plasma, or in both, an esterase acts on the vitamin, and the outcome is a fairly constant plasma level of the order of 1 i.u. (0.3 μ g.) free vitamin A per ml. (4) A small but fairly constant amount of vitamin A is found in the pigment epithelium readily accessible to the retina. (5) In the process of dark adaptation the reddish pigment, rhodopsin (visual purple), accumulates in the rods of the retina, but the process is delayed when the blood level of vitamin A is low; there is then defective scotopic vision, that is to say vision in light of low intensity. (6) Exposure to bright light results in decomposition of rhodopsin in vivo but the pigment is regenerated in the dark. (7) In some freshwater fishes and amphibia rhodopsin is replaced by porphyropsin and vitamin A_1 by vitamin A_2 . The latter differs from the former in having one additional conjugated double bond in the substituted six-membered ring. (8) Photopic vision, which is vision in light of high

intensity, differs from scotopic vision in that there is discrimination of colour as well as of brightness. The physiological observations requiring chemical interpretation are much more complicated in photopic vision than in scotopic vision but as yet no key substance other than vitamin A can be suggested.

Rhodopsin and porphyropsin

The rods in the retina of the vertebrate eye are composed of inner and outer segments. The outer limb is easily detached mechanically, and although it has no nucleus it is rich in rhodopsin. Suitably treated rod suspensions yield solutions of rhodopsin in 1 % aqueous digitonin. Rhodopsin is a conjugated protein and according to one careful experiment its molecular weight is near 270,000. In solution its absorption curve shows λ_{max} 500 m μ ., with weaker selective absorption near 340–350 m μ ., and stronger selective absorption near 275 m μ . due to aromatic acids in the protein. For porphyropsin, which in some fish and amphibian eyes plays the same part as rhodopsin, λ_{max} occurs near 522 m μ .

In isolated retinas, the final product obtained after exposure to light is vitamin A₁ from rhodopsin and vitamin A₂ from porphyropsin. The process can occur also in solutions of rhodopsin which have not been treated with alum, and in a cell-free brei from ox retinas. Exposure to light of preparations of rod outer segments, or of rhodopsin solutions prepared therefrom, results not in vitamin A₁ or A₂ but in retinene₁ or retinene₂. If, however, the rod outer segments are suspended in a watery extract of retinas, vitamin A can again be formed as a result of irradiation with visible light.

Retinene₁ and retinene₂

These two substances, derived photochemically from the prosthetic groupings of rhodopsin and porphyropsin, respectively, may be extracted by means of chloroform. They are characterized by absorption maxima in chloroform at 385 and 405 m μ . respectively (cf. vitamin A₁ 326 m μ ., vitamin A₂ 350 and 286 m μ .). With the antimony-trichloride reagent (SbCl₃ in CHCl₃) they show blue-green colours with λ_{max} 664 and 705 m μ . respectively (cf. vitamin A₁ 620 m μ ., vitamin A₂ 695 m μ .). The amounts of them obtainable from retinas are exceedingly small, and only their very high molecular extinction coefficients account for their spectroscopic detection in such low concentration. Controlled oxidation of vitamins A₁ and A₂ yields the corresponding aldehydes C₂₀H₂₈O and C₂₀H₂₆O. Both have been obtained pure and crystalline, and show the properties of retinene₁ and retinene₂ respectively. By the Oppenauer reaction, retinene₁ has been obtained from vitamin A₁, and under certain conditions a substance C₂₀H₂₆O, isomeric with retinene₂ and very closely resembling it, appears instead.

The conversion of vitamin A aldehyde to vitamin A is a reversible process involving a retinene reductase, vitamin A dehydrogenase. The apo-enzyme extracted from cattle retinas works equally well on the two aldehydes; it requires coenzyme I. The hydrogen donor used experimentally was fructose 1:6-diphosphate which presumably is first split to yield 3-glyceraldehyde phosphate.

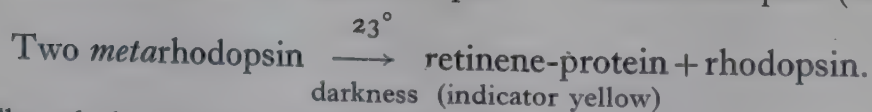
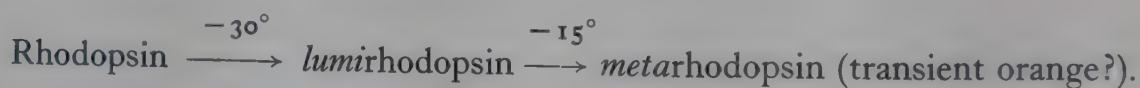
Photochemistry of rhodopsin

The initial photochemical change in the bleaching of rhodopsin by light is followed by a series of reactions in the dark. The latter can be greatly delayed if the irradiation is carried out at quite low temperature. At -78° an orange-coloured substance, transient orange, is formed ($\lambda_{\max}$ c. 490 m μ .), but on warming to room temperature indicator yellow is formed, and this is the immediate precursor of retinene₁.

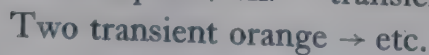
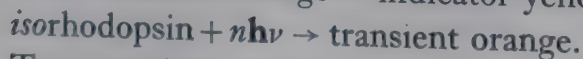
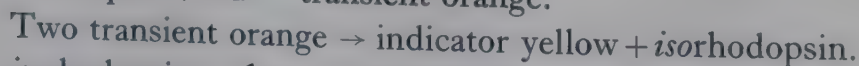
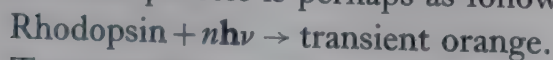
Vitamin A aldehyde prepared in vitro combines with many amines (e.g. CH₃NH₂), amino-acids and proteins to yield solutions with $\lambda_{\max}$ 365 m μ . in alkaline media, and 440 m μ . in acid media. These maxima agree with those of natural indicator yellow and the absorption curves are entirely analogous to those of indicator yellow.

Solutions of rhodopsin prepared in a dim red light may be frozen at -78° and thereupon exposed to intense white light. The pink solid then becomes orange in colour. The material is then allowed to come to equilibrium at room temperature in the dark (about 1 hr.). Almost exactly half the original absorption at 500 m μ . is restored and about half the maximum amount of indicator yellow is formed. For the regenerated rhodopsin (*isorhodopsin*) $\lambda_{\max}$ is slightly, but definitely, below 500 m μ .

The irradiation at -78° is now repeated and a second regeneration at room temperature is allowed to take place. Again the amount of indicator yellow increases and the absorption of the regenerated '*isorhodopsin*' falls by half to 25 % of the original. The whole process may be repeated several times, and the value of $\lambda_{\max}$ remains constant at 492 m μ . for the regenerated material. At intermediate temperatures (-45° , -15° etc.) rather more complicated results are obtained:



The whole process is perhaps as follows:



It is clear that to account for the fact that materials absorbing selectively near 500 m μ . are obtained from retinene, it is necessary to invoke either the action of free radicals or of compounds in which two C₂₀ molecules are attached so as to maintain conjugation. Structures consistent with the properties of rhodopsin, transient orange, and indicator yellow have been put forward.

There are weighty reasons for thinking that in normal scotopic vision the act of absorbing light results in electron transfer along the chain of conjugated double bonds to the retinal end-organ, and that bleaching by light is not necessarily a normal and integral part of the perception of dim light. The photochemistry, which is a key to investigation, may not reproduce any physiological process except near the threshold between scotopic and photopic vision.

Photopic vision

The scotopic sensitivity curve with $\lambda_{\text{max.}}$ 500 m μ . agrees well with the absorption spectrum of rhodopsin. The photopic action spectrum with $\lambda_{\text{max.}}$ 560 m μ . has been taken to imply the existence of iodopsin analogous to rhodopsin. The electrophysiological studies of Granit reveal action spectra corresponding with his concept of dominators and modulators. A dominator is a sensory mechanism, scotopic or photopic, characterized by a broad sensitivity curve extending over a wide range of wave-lengths; the difference between the principal dominators accounts for the Purkinje shift. Modulators have a much narrower response curve and their action spectra fall into groups situated in three spectral regions, 440–470, 520–540 and 580–600 m μ . There is also a modulator near 500 m μ . Their narrow action spectra make the modulators peculiarly suitable as mediators of colour vision.

There is at present great activity in the study of colour vision, and it is very difficult for chemists to decide which physiological observations are likely to provide 'labels' for chemical substances and which are not. Thus iodopsin may be as distinct a substance as rhodopsin or it may correspond with the summation of the absorption spectra of three or more modulators.

The modulators with their narrow-banded action spectra bear a certain resemblance to the unstable absorbing entities present in the chloroform solutions of the product of the antimony-trichloride reaction. A vast excess of antimony trichloride is necessary to ensure the stability of the colours, and even then it is not great. The two narrow bands for vitamin A₁ (620 m μ .) and retinene₁ (664 m μ .) are both accompanied by satellite absorption.

Vitamins A₁ and A₂ and the retinenes give rise to coloured solutions with concentrated sulphuric or phosphoric acid. The absorption spectra of such solutions exhibit narrow bands with maxima agreeing fairly closely with those of Granit's modulators. The existence of these modulator analogues makes it necessary to explore the possibility that photopic as well as scotopic vision requires vitamin A as an indispensable part of the chemical system.

Attempts to prove the existence of iodopsin as an independent entity have not been successful enough to compel assent, but both Wald and Bliss have provided indirect evidence for the presence of such a pigment in the cones. The material is not very stable but it seems clear enough that it can produce retinene. Nevertheless, it is not yet proved that retinene and vitamin A have a central position in photopic vision, but there is no other working hypothesis.

The biological activity of vitamin A depends in the last resort on the chain of conjugated double bonds from which derive (a) electron mobility, (b) ease in effecting the change in vivo of $\text{RCH}_2\text{OH} \rightleftharpoons \text{RCHO}$, and (c) ready reactivity of the $-\text{CHO}$ group with NH_2 or other groups in proteins.

Many questions remain unanswered, such as the nature of the collection of enzymes in the rod outer segments, the chemical constitution of rhodopsin, whether, for instance, it contains phospholipins and nucleoproteins, the mechanism of stabilizing visual pigments, and the role of trace metals in eye tissues.

The evolutionary significance of the change from porphyropsin to rhodopsin, which occurs in some species of amphibia on metamorphosis, is an important and most interesting field of investigation.

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A full list of references to original papers on which the above summary is based would occupy much space. Most of the earlier work is referred to by Morton (*Rep. Progr. Chem.* 1950, **46**, 244). R. Granit (*Annu. Rev. Physiol.* 1950, **12**, 485) reviews recent work on the physiology of vision, and G. Wald (*Biochim. Biophys. Acta*, 1950, **4**, 215) discusses the interconversion of the retinenes and vitamins A in vitro. The following refer to the most recent work:

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Collins, F. D. & Morton, R. A. (1950). *Biochem. J.* **47**, 3.
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Standardization and Requirement of Vitamin A

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Introduction

The standardization of vitamin A and the requirement for vitamin A are two subjects not very closely allied but they have both received special attention from the Vitamin A Subcommittee of the Medical Research Council's Accessory Food Factors Committee of which I have been the Secretary ever since it was first set up to deal with the standardization of vitamin A. Most of what is now said refers to the joint work of the members of that Subcommittee. There are two stories to be told and both are longer than there is space for, so only that of standardization will be told in full.

It was in 1931 that the Health Organization of the League of Nations held its first conference, aimed at setting up international standards for certain of the vitamins, for at that time there were no methods except biological methods for testing any of the then known vitamins. It was essential to set up standard substances to act as stable yardsticks against which materials could be tested for their potency and to define a fixed amount of the standard substance whose unchanging activity should be the international unit.

Standardization

Establishment of the carotene standard

At this first conference carotene was set up as international standard for vitamin A, and it retained this position, not unassailed, until 1949 (Permanent Commission on Biological Standardization: League of Nations, 1931). Indeed it has long been under heavy criticism, mainly on the fundamental ground that it is not vitamin A. Such a criticism had at first little sense, because no satisfactory form of vitamin A itself was available, but by 1939 pure esters of vitamin A had been prepared, and the stage was all set to propose vitamin A β -naphthoate as international standard. These plans were

frustrated by the war and the carotene standard received a new lease of 10 years of life during which it has been more heavily criticized than ever.

The carotene standard of 1931 was a crystalline mixture of carotenes, and the vitamin A activity of 1 $\mu\text{g.}$ of it was the unit of vitamin A. At a second International Conference in 1934 (Permanent Commission on Biological Standardization: League of Nations, 1934), the preparation of mixed carotenes was changed to one of pure crystalline β -carotene. The international unit had to be changed correspondingly and, by collaborative biological test, it was established that the amount of the pure substance that had the same activity as 1 $\mu\text{g.}$ of the mixture was 0.6 $\mu\text{g.}$ It is very important to maintain the continuity of any international unit and the activity of 0.6 $\mu\text{g.}$ was accordingly adopted for the new international unit.

Whatever criticisms have been made against the β -carotene standard, it has to be granted that it has always behaved very well as a stable replaceable substance, which is an essential property for a standard. During the war the supply of it became exhausted, and a fresh preparation was made. It was tested against the original preparation at the National Physical Laboratory and the two curves of spectral absorption were almost exactly superimposed on one another.

Cod-liver oil as standard for vitamin A

The non-existence of any suitable preparation of preformed vitamin A was given as the reason for making carotene the standard, and this was true though vitamin A was available in fish-liver oils and concentrates. They were not generally regarded as suitable, but the International Conference of 1934 decided that it would be advisable to have a sample of cod-liver oil as a subsidiary standard. The United States Pharmacopoeia Advisory Commission had some time before issued a sample of Reference Cod-Liver Oil to be used in the United States as a standard, so the Board of Trustees of the United States Pharmacopoeia were asked whether they would place a quantity of their Reference Oil at the disposal of the Health Organization of the League of Nations. They most kindly and willingly consented to do so. The Reference Oil was tested in twelve laboratories and the value ascribed to it was 3000 i.u./g. The U.S.P. unit in terms of the cod-liver oil was to have the same activity as the international unit of β -carotene.

Unfortunately, cod-liver oil really was more unsuitable as an international standard than had even been supposed, and its adoption introduced a nigger into the wood pile who was not finally liquidated until the belated but felicitous International Conference of 1949.

Whether the U.S.P. Reference Oil never really possessed the value of 3000 i.u./g. found for it, or whether it early lost part of that value, it is not now possible to say, but its overvaluation introduced an additional complication into the problem of the conversion factor, to which it is now necessary to turn.

Conversion factor for vitamin A

The conversion factor for vitamin A is the number that relates the spectroscopic readings to the results of biological tests expressed in international units. In considering

the conversion of carotene into vitamin A, it is important to distinguish which kind of conversion is meant at the moment of discussion. Superficially and qualitatively the two expressions mean something quite different, but fundamentally and quantitatively the problem of the conversion of spectroscopic readings into international units is the problem of the quantitative conversion of carotene to vitamin A.

Although biological standardization may be necessary for a substance, there goes on all the time the battle to establish some physical or chemical means of measuring it so that the biological standard may be eliminated. This struggle is carried on very strongly by the commercial firms in their anxiety to rid themselves of the cumbrous and expensive procedure of biological testing. Vitamin A is bought and sold in terms of international units. All commercial firms dealing in vitamin A would like to use the physical method of determining vitamin A content if they could trust it, but sellers would like a high conversion factor, seeming to give them a large number of international units, while buyers would like a low conversion factor, seeming to give them a small number of international units, for the identical article.

The report of the 1931 International Conference says nothing at all about any spectrophotometric test for vitamin A. The report of the 1934 Conference makes the guarded statement 'It has been found that, within certain defined conditions, measurement of the coefficient of absorption (E) at 328 m μ . affords a reliable method for measuring the vitamin A content of liver oils and concentrates. As a means of converting values obtained for $E_{1\text{ cm.}}^{1\%}$ 328 m μ . into a figure representing the International Units of Vitamin A per gramme of the material examined, the factor 1600 is recommended for adoption' (Permanent Commission on Biological Standardization: League of Nations, 1934).

When we of the Vitamin A Subcommittee look back on the basis upon which that factor, 1600, was founded, we have to feel deeply thankful for our good luck. The slender evidence for it is presented by Hume & Chick (1935). I believe the figure was arrived at largely by Prof. R. A. Morton and must have been almost inspirational from the tough way in which it has held its ground, and still holds it for certain materials and with certain necessary qualifications. The adoption by the International Conference of the value, 1600, was provisional, and after the Conference it was the immediate task of the Vitamin A Subcommittee to try to put the value for the conversion factor on a sounder basis.

The three collaborative trials

For a long time at any rate, for various inherent reasons, the biological test for vitamin A yielded results that were subject to greater variations than the results of other biological tests. It was consequently necessary to enrol as many laboratories as possible, and use as many animals as possible, to carry out the tests. Three main trials were made in each of which some dozen laboratories participated, the commercial firms taking an equal part with the members of the Vitamin A Subcommittee.

When the first test (Hume, 1937) was made, ideas were current that a conversion factor of about 2000 was attached to some cod- and halibut-liver oils, whereas one more like 1000 was associated with some concentrates. The materials chosen for test were,

therefore, a rich halibut-liver oil and a concentrate of its unsaponifiable fraction. The respective conversion factors obtained were 1470 for the halibut-liver oil, and about 1000 for the concentrate. The value 1470 did not disagree too badly with the provisional figure of 1600 for oils, and provided no basis for changing it, and that of 1000 was in accordance with current experience of concentrates. When the results of the tests had been fully analysed statistically the value for the halibut-liver oil was found to be 1570 instead of 1470, and the agreement with the value of 1600 provisionally recommended by the International Conference of 1934 was striking. Fortunately, in planning our experiment, we had arranged for spectrophotometric control of all the vitamin A solutions used in the biological experiments, and residues were returned by all the participants at the end of their feeding tests. This was a great advance, and was a procedure that we always afterwards followed. The spectrophotometric tests made it quite clear that the concentrate of unsaponifiable fraction was unstable and had deteriorated during the biological tests. The results of the biological tests would, therefore, give too low a conversion factor when referred to the spectroscopic reading made at the beginning of the whole investigation. The set of discrepant conversion factors round about 1000 which depended on the instability of the alcohol form of vitamin A was thus disposed of.

Meanwhile, in the United States, a value of 2000 for the conversion factor was taking stronger and stronger hold. It was the habit there to use the U.S.P. Reference Oil as standard of reference more often than the international β -carotene standard, and it appeared that a conversion factor of about 2000 was commonly obtained when rich oils were tested against the Reference Oil. The suspicion was gaining ground in England that the Reference Oil was overvalued and did not really possess its declared potency of 3000 i.u./g. Whispers to the same effect came from America also. If it were indeed so, oils tested against it would be overvalued too, and the number relating their value in international units to the spectrophotometric reading would be too large.

The Vitamin A Subcommittee decided, therefore, to make a collaborative test of the U.S.P. Reference Cod-Liver Oil against the β -carotene standard. The same procedure was followed as before, and the value obtained on the unsaponifiable fraction of the oil was 2619 i.u./g. instead of the declared value of 3000. All substances tested against it would, therefore, be overvalued by rather more than 12 %. The conversion factor found was 1820, and though less than 2000 was still high (Hume, 1939).

A further complication ensued from the overvaluation of the U.S.P. Reference Oil. The U.S.P. unit was defined in terms of the Reference Oil and was officially identical in value with the international unit. In fact, the overvaluing of the Reference Oil meant that the U.S.P. unit was worth about 12 or 13 % less than the international unit.

The longer such a state of affairs went on, the more desperately involved the situation must become, between usage in Great Britain and in the United States. When the solid esters of vitamin A first described by Hamano (1935, 1937) were prepared in the pure state by Mead (1939), the possibility appeared of solving the whole complicated situation by changing the international standard from β -carotene to an ester of vitamin A. The third collaborative test was, therefore, started in 1939 to make the comparison between vitamin A β -naphthoate and the international standard β -carotene.

The tests were completed with difficulty and the international conference with a view to which they were made was never held. The conversion factor obtained was 1770 (Hume, 1943). The values found in the two previous collaborative experiments of 1570 and 1820 had been regarded as significantly different but when the intermediate value of 1770 was obtained in the third experiment, it was considered that the three did not differ significantly and could be pooled to give the value of 1740.

The result was published during the war but, since no international conference could be held, the original provisional figure of 1600 continued to be used in the United Kingdom while that of 2000 was being used in the United States. Britain became a large purchaser of vitamin A from the United States, for inclusion in the ration margarine, and the two different conversion factors were a constant source of irritation and embarrassment to the Ministry of Food. From the other side of the Atlantic, it appeared that vitamin A was lost in the Atlantic, for it started out labelled with a certain value in U.S.P. units, but on arrival in England was labelled with a smaller number of international units in spite of the fact that no difference between the value of these two units could be officially recognized in the United States. Goodwill overcame the difficulties somehow and we got our vitaminized margarine.

When the war was over, it was more clear than ever that some drastic change would be needed to solve the complicated muddle into which the whole question of vitamin A standardization had got. It had not been possible during the war to do any more work in the United Kingdom towards devising a new standard, but those who had to evaluate the vitamin A concentrates for margarine derived an enormous amount of valuable experience through the mass of material which they had to handle. Prof. Morton's laboratory was able to elaborate the spectrophotometric method of testing and the workers for Messrs Lever Bros. and Unilever Ltd. gave much attention to the biological test.

Vitamin A acetate as international standard for vitamin A

Meanwhile, in the United States, it had been possible to continue research on the preparation of pure esters of vitamin A. Of those prepared the acetate seemed more suitable than the β -naphthoate, and the most suitable to act as a new international standard, and a collaborative study of the same sort as those made by the Vitamin A Subcommittee was organized and carried out for the United States Pharmacopoeia Vitamin Advisory Board. Biological tests of the acetate against β -carotene were made in some twelve laboratories, with a simultaneous spectrophotometric test and a statistical analysis of all the results. As the result of these tests, the United States Pharmacopoeia Vitamin Advisory Board announced a solution of vitamin A acetate as their new Reference Preparation to replace the Reference Cod-Liver Oil. The amount of the acetate found equivalent to 1 i.u. (0.6 μ g.) of β -carotene was 0.344 μ g. (or 0.3 μ g. vitamin A alcohol), so that 1 g. of the pure substance must contain

$$\frac{1}{0.3} \times 10^6 = 3.3 \text{ million i.u.}$$

The spectrophotometric E value, $E_{1\text{cm}}^{1\%}$ at 328 m μ ., is accepted as just about 1750

in certain solvents. From our definition of the conversion factor then, the value for it must be 3.3 million divided by 1750 which is just about 1900. The actual figure mentioned by the U.S.P. Vitamin Advisory Board was 1894.

About the time that the work on vitamin A acetate for the U.S.P. Vitamin Advisory Board was completed, the World Health Organization was being set up, linked for a time with the old League of Nations Health Organization by an Interim Commission.

The Interim Commission decided to hold an international conference on biological standardization and, through the Department of Biological Standardization at the National Institute for Medical Research, the Accessory Food Factors Committee was asked to prepare a brief on vitamins A and D.

To us of the Vitamin A Subcommittee it appeared that the devising and testing of the new standard had been done for us in the United States and that we need not carry out any further tests ourselves, if we could be allowed to inspect the details of the United States tests. We could not conscientiously recommend the results for adoption unless we had been allowed to satisfy ourselves of their statistical validity. This opportunity was obtained for us by Dr E. M. Nelson of the Federal Security Agency, Food and Drugs Administration in Washington, at whose request Dr E. Fullerton Cook, Chairman of the U.S. Pharmacopoeia Vitamin Advisory Board, placed the data in our hands. They were examined for us by Dr J. O. Irwin who has always been the statistician associated with the work of the Vitamin A Subcommittee. He reported favourably on the statistical treatment of the results and we were greatly relieved to feel that we had not to undertake yet another collaborative investigation.

The relation of the conversion factors found in the United Kingdom to that found for vitamin A acetate

The conversion factor found for the acetate was 1900, and it is necessary to consider how far this value is compatible with the factor of 1600 provisionally recommended by the International Conference of 1934, and with the factors of 1570, 1820 and 1770 found by the Vitamin A Subcommittee in their three collaborative experiments.

Nothing has so far been said about the question of irrelevant absorption which is one of the major factors complicating the spectrophotometric measurement of vitamin A. In this country it has essentially been the field of Prof. R. A. Morton, first in collaboration with Dr J. R. Edisbury and later with Mr T. W. Goodwin and Dr A. L. Stubbs. They have worked on it continuously and have brought the difficulty well under control.

The irrelevant absorption is the spectral absorption at or near the maximum absorption of vitamin A, which is caused by the presence of other substances devoid of vitamin A activity. A large part of it can be eliminated by saponification. It does not, of course, affect pure substances, and is more important the greater its magnitude in relation to the amount of vitamin A present. The more it inflates the spectrophotometric reading in relation to the biological value, the smaller will be the conversion factor.

Of the three conversion factors, 1570, 1820 and 1770, obtained by the vitamin A Subcommittee, the last was with a pure substance, vitamin A β -naphthoate, and the second was with an unsaponifiable fraction; only the first, the lowest, was with a whole

oil. The first, therefore, was the only one in which irrelevant absorption might have played a part. By great good fortune, a portion of the original halibut-liver oil used for the first test was still in existence in satisfactory cold storage, and Prof. Morton was able to retest it spectrophotometrically, after eliminating the irrelevant absorption. The corrected value thus obtained for the conversion factor was 1830 instead of 1570 (Morton & Stubbs, 1947).

In preparation for the International Conference by that time definitely announced by the Interim Commission, the Vitamin A Subcommittee asked Dr Irwin to examine the original data and combine these three conversion factors and the U.S. Pharmacopoeia value of 1894 in one figure. He did so and the value he found was 1837 with fiducial limits at $P=0.99$ of 94–106 %. Any value between 1800 and 1900 would thus be possible.

On both sides of the Atlantic, the data were thus all ready for presentation to the International Conference.

The World Health Organization finally came into being in succession to the Interim Commission, and its Expert Committee on Biological Standardization created a Subcommittee on Fat-Soluble Vitamins. The Subcommittee met in London on 26–29 April 1949. With Sir Edward Mellanby in the Chair, Dr R. Gautier as Secretary and many other familiar faces, the Conference was in full continuity with its predecessors of 1931 and 1934. It was a most felicitous Conference which reached its decisions with complete unanimity.

The United States had already grasped its worst nettle when it made the new U.S. Pharmacopoeia unit based on vitamin A acetate equal to the potency of 0.6 $\mu\text{g.}$ of β -carotene instead of making it equal to one unit of the old, overvalued Reference Oil. This must have meant writing down the value of great stocks of vitamin A. Nothing, therefore, stood in the way of accepting the acetate and the new U.S.P. unit as international standard and international unit, respectively.

If the value of 0.34 $\mu\text{g.}$ was accepted as the value for the unit, there could be no argument about the conversion factor because the spectrophotometric value for pure vitamin A acetate was generally accepted. The conversion factor had to be 1900, and there was no longer anything in the British results to conflict with it, as long as irrelevant absorption was ruled out.

The Expert Subcommittee was, therefore, able in full harmony to recommend the acceptance of vitamin A acetate as international standard for vitamin A, with the activity of 0.34 $\mu\text{g.}$ of it, or 0.3 $\mu\text{g.}$ of vitamin A alcohol, as the unit, and 1900 as the conversion factor attached to it. The serviceable old β -carotene standard was retained for biological estimations of carotenoids. These recommendations have since been accepted by the superior bodies (World Health Organization, 1949) and are in the press.

The conversion of carotene to vitamin A

Out of this long research on the magnitude of the conversion factor comes clearly a conclusion of fundamental importance.

If 0.3 $\mu\text{g.}$ of vitamin A has the same activity as 0.6 $\mu\text{g.}$ of β -carotene, which is the essential biological result, then the molecule of β -carotene does not split into two

molecules of vitamin A as was long supposed, but produces only one. At any rate that is the average result of all the four collaborative researches which have been mentioned. There is some evidence that conditions exist in which it is not always so, but in the conditions of the collaborative experiments it was so.

Relative value of the biological and spectrophotometric tests

At times in these 20 years, controversies have blown up that the biological test or the spectrophotometric test was no use at all, but of course the truth is that both were essential.

It is true that the biological test is not at all accurate but the spectrophotometric test is valueless as a test of biological activity if it is not checked all along the line by the biological test.

To attain any degree of accuracy the biological test requires so clumsily many animals that it is the aim of all to eliminate it in favour of the spectrophotometric test, wherever it has been established that that can reliably be done.

Now that the dust of battle has died down, it is possible to see the various subjects of conflict in better proportion.

Other conversion factors proposed

The story has been confined to the things that happened in Great Britain and the United States. Other contributions have been made, and claims have been set up for other conversion factors. There is only one to which the temptation to refer is irresistible, and that is the claim for a conversion factor of 3000 for the German vitamin A concentrate, Vogan. Such a conversion factor was of course very favourable in foreign trade for Nazi economic policy. It is probably better to mention no names of Germans. Some are perhaps fortunately dead and the whole truth is not known.

The claim for the very high conversion factor for Vogan, which was a fish-liver oil concentrate, was based on the fact that the vitamin A in it was mainly in the ester form. The claim was supported by a large, consistent, published series of animal experiments made with rats against a standard at various times of the year. One could not see what could be wrong with them. One fact, however, was suspicious. A test on the U.S. Pharmacopoeia Reference Oil in which there was vitamin A in the ester form did not yield a conversion factor of 3000. It could not; the facts or approximate facts about the Reference Oil were too well known.

During the war the Swiss Professor Edelbacher challenged the value claimed for Vogan and the Germans were forced to retest it. They did so and found a conversion factor of about 2000 and that the Vogan preparation sold commercially was correspondingly overvalued. The actual value found in the biological tests was 77,000 i.u./g. instead of the 120,000 i.u. claimed. How the original results were obtained has not been explained. Use of a deteriorated carotene standard might produce the effect, but the explanation does not much matter any longer, now the claim is no longer made, and as long as it is known that 0.64 is the factor by which all results obtained against Vogan as standard should be scaled down.

Requirement of vitamin A

Another main preoccupation of the Vitamin A Subcommittee was a large-scale experiment which attempted to determine the human requirement of vitamin A.

The research was undertaken in response to a request in 1940 made to the Medical Research Council by Sir Jack Drummond, then Chief Scientific Adviser to the Ministry of Food, for more accurate information than was available as to the human requirement in terms of vitamin A and of β -carotene. When the request came it was obvious that a human experiment was the only way to find an answer, but a supply of human subjects sufficient for the purpose seemed a day-dream. Then it was learnt that such a group existed. It had been built up by Dr Kenneth Mellanby in Sheffield from conscientious objector volunteers, particularly for research on scabies. A team of investigators was formed including many more than the Vitamin A Subcommittee. At first Dr Kenneth Mellanby and Prof. H. A. Krebs were in charge on the spot. Then Dr Mellanby went abroad and Prof. Krebs took sole charge in Sheffield with the Vitamin A Subcommittee and the rest of the team meeting there about once a month. It sounds a cumbersome way of conducting an experiment but it worked very well. The course of the experiment was so unpredictable from the first, the prime unknown being the length of time it would take to deplete the men on a diet deficient in vitamin A, that it was highly satisfactory to be able to steer the experiment by constantly renewed general discussion, with the opportunity of considering the nutritional state of each of the twenty-three individuals separately every month. Some of them were receiving prophylactic doses of vitamin A or β -carotene, while others were undergoing depletion with a view to quantitative, curative dosing.

The account of the experiment has been published in full in a Medical Research Council green report (Hume & Krebs 1949) and is accessible to everyone, so that a little brief comment is all that is necessary.

It must be remembered that very few of the individuals ever became sufficiently depleted to show unmistakable signs of change on dosing. No unequivocal signs of depletion appeared within the 1st year. This was in itself a valuable result since previous evidence as to depletion times was conflicting. It harmonizes, however, with the general experience in western Europe during the war, where authentic vitamin A deficiency was scarcely seen, though circumstances must have arisen in which individuals had to subsist on their reserves for certain periods. The existence of such large reserves in the experimental subjects meant that there was only a very small number of persons sufficiently depleted for test dosing.

Requirement in terms of vitamin A. For vitamin A the evidence obtained from a curative test, from prophylactic tests, and from the values found for the liver reserves of persons dying through accident or other violence in Great Britain, led to the conclusion that 1300 i.u. vitamin A could be regarded as the minimum daily protective dose for a healthy adult. To allow for individual variation and create a margin of safety it was recommended that 2500 i.u. vitamin A be put forward as the minimum daily requirement for healthy adults. The procedure of doubling the value for the minimum protective dose to secure the requirement has been criticized and is certainly open to criticism. Some such margin has, however, to be created, and it is a matter of judge-

ment rather than of facts how that should be done. No pronouncement based on the results of the Sheffield experiment can of course be made for the requirements of vitamin A or of carotene for any other class of individuals than healthy adults.

Requirement in terms of β -carotene. For β -carotene the assessment of the requirement from the evidence is more difficult because the absorption of the dose given is incomplete to an extent varying with the nature of the vehicle in which it is given.

From prophylactic and curative tests it was concluded that the minimum protective dose, on the assumption that all the β -carotene administered was absorbed, must lie between 1250 and 1900 i.u. daily for a healthy adult. On the same supposition a figure of 1500 i.u. daily was, therefore, recommended. For the same reasons as were given in connexion with vitamin A, this value was doubled so that the figure 3000 might be regarded as generally applicable to healthy adults.

Since, however, the figure 3000 supposes that all the β -carotene is absorbed, which is really not so, a factor has to be applied to increase the intake to the amount necessary to secure that the body actually absorbs 3000 i.u. daily. Absorption experiments were made on the Sheffield volunteers with a few foods, which established roughly that the amounts of carotene that had to be offered in order that 3000 i.u. should be absorbed were from carrots, boiled, sliced or made into puree 12,000, but if the carrots were homogenized only 5500, from cabbage or spinach 7500, and from oil or fat 4000. It is thus obviously highly undesirable to state the daily requirement of β -carotene as any one figure though, if it is essential to have one figure, the compromise value of 7500, three times the vitamin A requirement, was reluctantly put forward.

The values suggested from their experiment by the Vitamin A Subcommittee do not agree too badly with the value of 5000 put forward by the National Research Council (1948) in the United States, with the note attached that the allowance is 'based on the premise that approximately two-thirds of the vitamin A value of the average diet in this country is contributed by carotene and that carotene has half or less than half the value of vitamin A'. If, on this basis, the mixed value of 5000 is recalculated as entirely vitamin A, the value obtained is 3250 or less, and if it is recalculated as all β -carotene the value is 6500 or more. These figures do not differ too excessively from the Vitamin A Subcommittee's suggestions of 2500 and 7500.

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Site of Conversion of Carotene to Vitamin A

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The liver has for a long time been considered as the main site of metabolic activity and it seemed natural that, when Moore (1930) demonstrated that carotene is converted in the animal body to vitamin A which is then stored in the liver, he assumed (Moore, 1931) that it is in the liver that conversion takes place. This view was generally held for the next 15 years though the supporting experimental evidence, ably summarized by Glover, Goodwin & Morton (1948*b*), was neither satisfactory nor conclusive.

The almost complete absence of carotenoids, even after a carotene meal, from the blood of some animals, notably the rat, the pig, the sheep and the goat, provided an important objection to the hepatic theory, since it failed to explain how in such animals the carotene taken up by the intestine would reach the liver. The possibility of a brief travel through the portal vein followed by an immediate and quantitative removal by the liver was discounted by the experiments of Goodwin, Dewar & Gregory (1946) and of Goodwin & Gregory (1948) who failed to detect carotene in the portal or systemic blood of rabbits, goats and sheep after administration of massive doses in various forms by mouth or directly into the true stomach or the duodenum, yet vitamin A similarly given appeared in the blood in large quantities.

Attempts to produce experimental proof turned mainly on the value of carotene when given parenterally, since the demonstration of its activity under such conditions would go far in support of the hepatic theory of conversion. The findings were conflicting and most of the experiments open to criticism (cf. Glover *et al.*, 1948*b*). More recently, however, Sexton, Mehl & Deuel (1946) produced clear-cut evidence that carotene introduced parenterally into vitamin A-deficient rats was not only not converted but accumulated in the liver without relieving the symptoms of deficiency. From these experiments Sexton *et al.* concluded that 'a possible site for the transformation of carotene to vitamin A might be in the intestinal wall'. This possibility had also occurred to some earlier observers. Thus Verzár & McDougall (1936) commented as follows on remarkable experiments from Drummond's laboratory: 'Drummond, Bell and Palmer (1935) have recently described in a patient with chylothorax the path of absorption of carotene and vitamin A. . . . Vitamin A was given as the free alcohol. During the passage through the intestinal wall all the vitamin A was esterified with fatty acids. Whether the carotene was also already transformed to vitamin A in the mucosa—as one might suppose—was not studied.' Nearly 10 years ago Popper & Greenberg (1941) observed by fluorescence microscopy the appearance of vitamin A in different sites of depleted rats and reported: 'The first fluorescence after feeding of carotene was seen in the intestine or in the Kupffer cells and then in the adjacent parts of the liver cells or in the endothelial cells of the renal cortex and lung or in the adrenal cortex. . . . Probably carotene is converted into vitamin A in one or all of these locations.'

The finding that the intestine of fish contained vitamin A in very high concentration (Edisbury, Morton, Simpkins & Lovern, 1938; Lovern, Morton & Ireland, 1939;

Lovern, Mead & Morton, 1939) and localized in the mucosa (Lovern & Morton, 1939) further emphasized the possible importance of this organ in the metabolism of the vitamin.

The intestine as site of conversion

Finally evidence for the intestinal conversion of carotene in higher vertebrates came independently and almost simultaneously from three different laboratories (Glover *et al.* 1947; Mattson, Mehl & Deuel, 1947; Wiese, Mehl & Deuel, 1947; Thompson, Ganguly & Kon, 1947). The preliminary reports from Liverpool and Shinfield were followed by full publications (Goodwin & Gregory, 1948; Glover *et al.* 1948*b*; Thompson, Ganguly & Kon, 1949). Further evidence came thence (Thompson, Braude, Cowie, Ganguly & Kon, 1949; Thompson, Coates & Kon, 1950; Coates, Thompson & Kon, 1950; Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950; Alexander & Goodwin, 1950) and from the Californian laboratory (Mattson, 1948; Cheng & Deuel, 1950). These original observations are supported by those obtained elsewhere (McCoord & Clausen, 1948; Krause & Pierce, 1948; Elliot, 1949; Klosterman, Bolin & Light, 1949; Swick, Grummer & Baumann, 1949; Stallcup & Herman, 1950).

However strongly this large body of evidence supports the intestine as the organ where conversion takes place, the evidence against the liver as another, or for that matter the only, active site is still only indirect. As far as we know all experiments but one with living animals were performed on those with unimpaired liver circulation, and in none was the liver removed. Even the valuable study of Krause & Pierce (1948), in which the hepatic circulation was blocked, cannot, on their own admission, be taken to settle the point. Likewise the decision does not rest with experiments *in vitro*, valuable though they may be, since the synthetic activity of the dead intestine or of intestinal tissues is not a determining guide to the chain of events in the living body.

The proof is based on the appearance of vitamin A outside the liver after a meal of carotene under conditions making it clear that it originated in the small intestine. Though overwhelmingly strong, the proof springs not from one crucial experiment, but derives from the sum-total of evidence. We think it more prudent, therefore, to treat it as circumstantial and to consider in reasonable sequence its component links. This summing up need be only brief, since more detailed discussion of most points will be found in the original papers, and especially in the two full reports from our laboratory published in this journal.

Validity of analytical methods used

As far as we are aware, the vitamin A appearing outside the liver, in the intestine or in the lymph flowing from it, has been identified only by chemical or physical means and biological proof is not yet available. However, in several instances the measurements either by the antimony-trichloride test (Glover *et al.* 1948*b*; Thompson, Ganguly & Kon, 1949; Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950) or by absorption in the ultraviolet (Glover *et al.* 1948*b*; Mattson, 1948; Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950) were preceded by careful chromatographic purification and the spectral absorption curve proved identical with that of vitamin A. Moreover,

the characteristic fluorescence in the ultraviolet was also observed (Mattson, 1948; Thompson, Ganguly & Kon, 1949; Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950); it was identical in a mixed chromatogram with that of vitamin A (Mattson, 1948).

Absence of stored vitamin A from intestine; appearance after meal of carotene

Experiments with rats (Mattson *et al.* 1947; Glover *et al.* 1948*b*; Thompson, Ganguly & Kon, 1949), chicks (Cheng & Deuel, 1950) and lambs (Klosterman *et al.* 1949) have shown that the intestine is not a storage place for vitamin A, and that it can be depleted of it in a few days in animals with large liver reserves. After a meal of carotene vitamin A promptly appeared in the small intestine of such animals and also of deficient rats (Mattson *et al.* 1947; Glover *et al.* 1948*b*; Thompson, Ganguly & Kon, 1949; Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950) and chicks (Thompson, Coates & Kon, 1950; Cheng & Deuel, 1950) but not in the stomach or large intestine (Glover *et al.* 1948*b*; Thompson, Ganguly & Kon, 1949). In the rat the peak of concentration was just proximal to the middle of the small intestine irrespective of the time interval between dosing and killing, whereas for carotene the peak shifted towards the large intestine with progress of time. The vitamin did not appear in that part of the intestine preceding the entrance of the common bile duct (Thompson, Ganguly & Kon, 1949; Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950).

Time relationships after a carotene meal

In all these experiments with deficient rats and chicks the appearance of vitamin A in the small intestine preceded that in any other site, and for a time exceeded the accumulation in the liver. Thus in our experience under favourable conditions vitamin A may appear in the intestine of previously depleted rats within 5 min. of their receiving carotene in colloidal or oily solution (Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950), yet never in numerous instances did we detect it in increased quantities in the blood or in the liver less than 1 hr. after the carotene meal. In the rat, only after 2 hr. the steady accumulation in the liver began to outstrip in quantity that found in the intestine (Thompson, Ganguly & Kon, 1949). In the chick this time lag was even more striking (Thompson, Coates & Kon, 1950; Cheng & Deuel, 1950).

Behaviour in the intestine of carotene, retinene and vitamin A alcohol and ester

Whether carotene, retinene (vitamin A aldehyde), or free or esterified vitamin A was given it was largely the ester form of vitamin A that appeared in the intestine, and in all instances the site of maximal concentration was the same (Glover *et al.* 1948*a*; Thompson, Ganguly & Kon, 1949; Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950). According to Glover *et al.* (1948*a*) vitamin A aldehyde is the first step in the transformation of carotene to vitamin A *in vivo*, and this view is supported by the remarkable oxidative fission of β -carotene to retinene by manganese dioxide recently reported by Meunier, Jouanneteau & Zwingelstein (1950).

Most, if not all, of the vitamin A appearing in the intestine is concentrated in the

wall (Mattson *et al.* 1947; Glover *et al.* 1948*b*; Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950). The earlier finding of appreciable quantities in the content by Thompson, Ganguly & Kon (1949) was due to a faulty technique.

Blood relationships after a carotene meal

We have already mentioned that Goodwin & Gregory (1948) failed to detect carotene in the portal or systemic blood of rabbits, goats and sheep after massive doses of carotene. The same is true for the pig (Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950) and was observed for the systemic blood of the rat by all those who used this animal. Moreover, observations with the rat (Thompson, Ganguly & Kon, 1949) and the pig (Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950) indicated that, in these animals at least, after a meal of carotene an increase in vitamin A ester preceded in the blood an increase in vitamin A alcohol. Since, normally, vitamin A alcohol as the circulatory form predominates in the blood, it seems reasonable to assume that the ester is on its way to the liver, its main storage place, from the intestine, where it was formed.

In the observations just mentioned with the pig the ester appeared simultaneously in the portal and systemic circulations, making it unlikely that it had gone directly into the portal route. The experience of Frazer (1946, 1948) with fat, and of Drummond *et al.* (1935), Popper & Volk (1944), Eden & Sellers (1948), Goodwin & Gregory (1948) and Thompson, Ganguly & Kon (1949), suggests that the transport occurs largely through the lymphatics. Experiments with lymph support this view.

Study of lymph

Examination of thoracic, and to an even greater extent of mesenteric, lymph has thrown much light on the problem of intestinal conversion of carotene. Cannulation of the thoracic duct in goats (Goodwin & Gregory, 1948) and of a mesenteric lymphatic in pigs (Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950) demonstrated the appearance in both sites after a meal of carotene of vitamin A, exclusively as ester, and in the pig the extent of the increase was remarkable. Alexander & Goodwin (1950) and Thompson, Braude, Coates, Cowie, Ganguly & Kon (1950) obtained similar results with the rat, and perhaps the experiments of the latter authors may be taken as yielding the most convincing proof, since they showed that by tapping and removing the lymph flowing from the intestine vitamin A was prevented from reaching the blood or the liver.

Blocking of liver circulation

In an important experiment Krause & Pierce (1948) ligated in the rat in a two-stage operation the portal vein, the hepatic artery and the common bile duct. The vitamin A content of the serum of such rats rose after a meal of carotene to an extent comparable with that observed with normal rats.

In vitro tests

In vitro tests have consisted mainly of incubation of intestines with carotene. It cannot be said that the findings are convincing. Though Wiese *et al.* (1948) and Glover

et al. (1948*b*) reported some formation of vitamin A under such conditions, the latter doubt the significance of their findings. Stallcup & Herman (1950) report positive results with calf intestine but even better efficiency with minced liver tissue.

We have so far failed in unpublished experiments done in collaboration with Drs Fisher and Parsons of Oxford to effect conversion of carotene by perfusion of surviving rat intestine by their technique (Fisher & Parsons, 1949-50).

Observations with several species of animals

The number of species that contributed to the evidence for intestinal conversion is already impressive. Work with rats, pigs, goats, rabbits, sheep and chicks has already been discussed. Elliott (1949) mentions briefly work with calves, and Klosterman *et al.* (1949) reported further experiments on sheep and Swick *et al.* (1949) on pigs.

Already 12 years ago Wagner (1939) claimed to have observed conversion of carotene to vitamin A in the intestine of baleen whales. Though we do not deny that whales possess this capacity we doubt whether they ever have the opportunity since, contrary to Wagner's statements, their food contains no carotene, but appreciable quantities of preformed vitamin A (Thompson, Ganguly & Kon, 1949; Kon & Thompson, 1949*a, b*; Batham, Fisher, Henry, Kon & Thompson, 1951).

The intestine as a metabolic site

The recent demonstration by Popják & Beeckmans (1950) that synthesis of cholesterol and of fatty acids takes place in the intestine adds force to the proof of intestinal conversion of carotene. In fact, its large surface and rich blood supply make it likely that the intestine is a seat of metabolic activity comparable with the liver.

Conclusion

It seems to us that on the basis of the evidence presented the conclusion is inevitable that β -carotene is transformed in the intestinal wall to vitamin A ester (probably through retinene) and that it is carried thence by the lymphatics to the blood stream and finally to the liver.

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Vitamin A Levels in Health and Disease

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The antimony-trichloride method for estimating vitamin A has now been available for nearly 25 years, and has been used to study the metabolism of the vitamin in man and animals by numerous workers all over the world. It is not surprising, therefore, that the wealth of literature is quite beyond compression into a short review, so that we cannot hope to outline more than a few of the main developments in the field, with emphasis on points in which we have been personally interested.

The levels of vitamin A in the blood plasma and tissues are influenced by numerous factors, including the rate of intake of vitamin A or carotene, the efficiency of absorption from the intestines, the storage of vitamin A in the liver and other tissues, its mobilization from the liver into the blood plasma, and its removal from the plasma by transfer to the tissues, by destruction, and sometimes by urinary excretion. The various stages in the absorption, mobilization and utilization of the vitamin, however, probably overlap considerably. Thus, it must not be concluded that the vitamin invariably passes through the liver before being transferred to other tissues.

Absorption

Preformed vitamin A is generally much more efficiently absorbed than carotene, although the disparity decreases, and possibly eventually disappears, at low levels of dosing. Numerous investigations have been made on the effect of massive doses of

vitamin A in raising its level in the blood plasma. In normal subjects a sharp rise, mainly in esterified forms of the vitamin, is observed 4-5 hr. after dosing, followed by a return to the resting value within 24 hr. 'Absorption' or 'tolerance' curves may be readily obtained, and have been used in America as a measure of the efficiency of fat absorption. Flattened curves are observed in diseases such as sprue, coeliac disease and infective hepatitis, in which the absorption of fat is affected. On the other hand, greatly elevated curves have been observed in nephrosis. Thus, Kagan, Thomas, Jordon & Abt (1950) found that children suffering from this disease not only had a very high resting level of vitamin A, but showed a much greater increase after dosing. In general the high levels of fat in the plasma, which are characteristic of nephrosis, tended to be associated with high concentrations of vitamin A, but the correlation was not close. The authors concluded that in nephrosis the power of the liver to absorb or utilize vitamin A is seriously impaired.

Numerous investigations have clearly demonstrated that in conditions with impaired fat absorption vitamin A is absorbed much more efficiently from aqueous emulsions made with the Tween group of non-ionic detergents than from oily solutions. Medicinal paraffin, although having little effect on the absorption of preformed vitamin A by normal subjects, is well known to interfere considerably with the absorption of carotene.

Storage and distribution

Experiments on rats and other animals have established that the body's main stores of vitamin A are usually located in the liver, being concentrated in both the liver cells and Kupffer cells. Recently experiments by Johnson & Baumann (1947*a, b*), however, have indicated that, in rats given doses of about 30 i.u. vitamin A or carotene daily, the amounts of vitamin A present in the kidneys exceed those in the liver. Eden & Moore (1950) have confirmed this surprising observation, and have drawn attention to the failure of the concentration of vitamin A in the kidneys to increase parallel with that in the liver when large doses of vitamin A were given. With liberal intakes of vitamin A substantial amounts appear in the lungs, adrenal glands and fat deposits.

Although more is known about the metabolism of vitamin A in the eye than in any other organ, it is important to realize that the retina normally contains only a very minute fraction of the total amount present in the body.

In studies of the distribution of vitamin A in the cells, fluorescence microscopy has given interesting results in the hands of Popper & Greenberg (1941) and others. In macroscopic examinations of the adipose tissue of rats we have observed yellow fluorescence under ultraviolet irradiation in animals given massive doses of vitamin A, but not in animals given inadequate doses. We have sometimes been able, however, to detect vitamin A by the antimony-trichloride reaction in specimens of fat which showed no yellow fluorescence. With some fluorescent tissues, moreover, the fluorescence was absent from the fat as extracted with ether, and remained associated with the residual tissues. In preliminary tests we succeeded in extracting the fluorescent substance from the residue with hot 50 % ethyl alcohol; from its distribution between ether and water it appeared to be water soluble.

Quantitative aspects. Since the early experiments by Moore (1930), it has been recognized that much larger doses of carotene or vitamin A are needed to cause storage of vitamin A in the livers of rats than to promote normal growth. Lemley, Brown, Bird & Emmett (1947) found that over the range of dosage which they investigated the percentage of vitamin stored increased with the magnitude of the dose up to an optimum point, after which there was a decline when very heavy doses were given.

Davies & Moore (1948) obtained similar results. In rats depleted of vitamin A single doses of 100 i.u. caused no storage in the liver. Larger doses were stored with increasing efficiency, until with 50,000 i.u. the efficiency of storage reached 85 %. With prolonged heavy dosing, however, the efficiency of storage decreased, presumably on account of saturation of the liver. At such levels the rats are in danger of hyper-*vitaminosis A*, characterized by broken bones, mainly in young animals, and sudden severe haemorrhage (see Moore & Wang, 1945).

The observation that low doses of vitamin A cause no storage in the liver lends colour to the report by Le Gallic (1947) that under certain circumstances the blood and tissues of albino rats may possess biological activity without containing significant amounts of vitamin A or carotene, as measured chemically. This conclusion, if confirmed, would suggest that vitamin A may exert its activity indirectly through transition into another substance, which might explain why the vitamin profoundly affects tissues, such as the mucous membranes and bones, in which its presence cannot be detected by chemical methods. In spite of numerous attempts, however, we have so far failed to confirm Le Gallic's observations.

Storage in different animals. The vitamin A reserves in different animals have been extensively investigated. The short list of values given in Table 1 may serve to demonstrate the extremely wide variations which are found between species. For each animal

Table 1. *Storage of vitamin A in the livers of various animals*

Species	Vitamin A content (i.u./g. liver)	Comment
Guinea-pig	10	Normal human range (median 320)
Pig	100	
Cow	150	
Rabbit	170	
Rat, wild	250	
Sheep	600	Toxic
Cod fish	2,000	
Sperm whale	4,400	
Bearded seal	13,000	
Polar bear	20,000	

↑

Range for
experimental rat

↓

the individual values also usually vary considerably, and most of the values given are means for groups. Since, moreover, the reserves are greatly influenced by the composition of the diet, they can be considered as typical only when the animal is adhering to its usual feeding habits. Thus, though a reserve in the liver of 250 i.u./g. might be considered as typical for wild rats in this country, values of from 0 to 20,000 i.u./g. may readily be obtained by suitable adjustment of the diets of rats kept in the laboratory.

For man the range in this country, as found for cases of accidental death, extends from 10 to about 2000 i.u./g. Moore (1949) found a median of about 320 i.u./g.

In spite of the influence of diet there are two clear instances in which animals on much the same diet have widely different reserves. Thus sheep at pasture have liver reserves of 600 i.u./g., compared with only 150 i.u. for cattle under the same conditions. Guinea-pigs, according to our estimations on animals kindly provided by Miss H. M. Bruce, have very much lower reserves than rabbits. Thus guinea-pigs, given a diet of cubes and greens, had only 10 i.u./g. compared with 170 i.u./g. for rabbits on the same regime. Since, in our experience, the livers of guinea-pigs can accumulate large reserves if large doses of the preformed vitamin are given, it would appear that the frequent occurrence of low reserves in this animal, which was first reported by Chevallier & Choron (1935), may be due to inefficiency in the conversion of carotene.

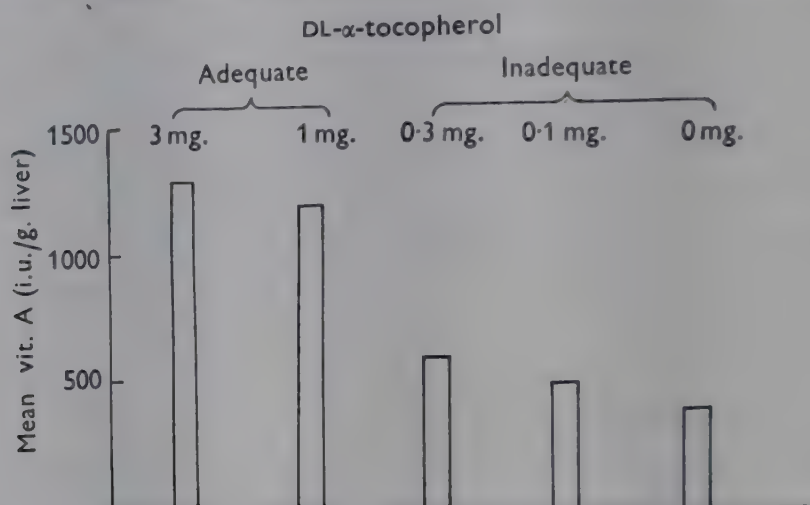


Fig. 1. Storage of vitamin A in the liver by female rats given 1000 i.u. vitamin A weekly for about 6 months, in conjunction with doses ranging from 0 to 3 mg. DL-α-tocopherol weekly.

The early experiments of Dann (1932) established that in rats vitamin A is transferred in limited amounts from the mother to the foetus, and the reserves in newborn animals have generally been found to be much lower than in adults. Thus the livers of calves at birth usually contain per g. about 10 i.u., those of lambs 30 i.u., and of human babies 20 i.u. A substantial contribution of vitamin A is provided by the colostrum.

Vitamin E and the storage of vitamin A in the rat. The effect of vitamin E deficiency on vitamin A reserves was first reported by Moore, Martin & Rajagopal (1939). Fig. 1 is based on observations by Moore (1940) in which pairs of female rats were kept for about 6 months on a basal diet with supplements of 1000 i.u. vitamin A and of from 0 to 3 mg. DL-α-tocopherol, weekly. With doses of 1 and 3 mg. of tocopherol, which were adequate judged by the colour of the uterus at autopsy, much higher stores of vitamin A were accumulated in the liver than with inadequate doses of from 0 to 0.3 mg.

Sex and the storage and distribution of vitamin A. Booth (Medical Research Council, 1949) and others have noticed that the vitamin A reserves in the livers of female rats are consistently higher than in those of males receiving the same diets. In recent experiments we have confirmed this conclusion at various levels of dosing. Fig. 2 shows the total amounts of vitamin A found in the livers and kidneys of male and female rats, expressed as percentages of the total amount ingested during the 4-5 weeks during

which the rats received the basal diet. It will be noticed that at all levels of dosing, except that of 10 i.u. daily, the reserves of the females exceeded those of the males. In confirmation of previous conclusions, moreover, the efficiency of storage tended in both sexes to rise with the magnitude of the doses given (see p. 121).

The effect of sex was perhaps more strikingly seen in the different distribution of the vitamin between the kidneys and liver. The average concentration in these organs at various levels of dosing has already been reported (Moore & Sharman, 1950) and

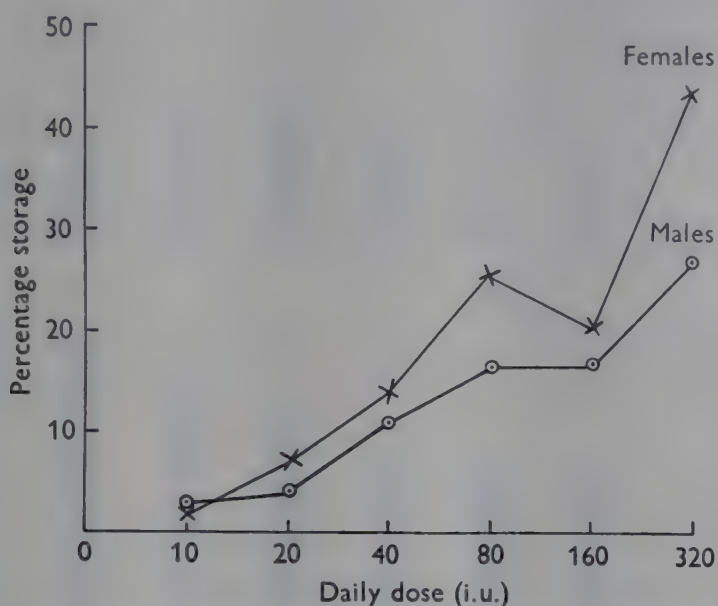


Fig. 2. Amount of vitamin A stored in the livers and kidneys of male and female rats, expressed as a percentage of the amount ingested.

showed that, contrary to observations on the liver, the kidneys of males consistently contained more vitamin A than those of females. Fig. 3 shows individual values for male and female animals that had received doses of 40 i.u. vitamin A daily for about 4 weeks. At this critical level of dosing the concentration of vitamin A in the kidneys of males was invariably greater than in their livers, whereas in the females the situation was reversed, the concentration in the liver being invariably much greater than in the kidneys.

Johnson & Baumann (1947*b*) have observed that vitamin A was transferred from the liver to the kidneys during rapid growth. It therefore remains to be decided to what extent the greater concentration of vitamin A in the kidneys of male rats can be explained by their growing more rapidly than females. Two preliminary experiments on the effect of sex hormones on the distribution of vitamin A may, therefore, be of interest.

In one experiment male rats, which possessed substantial initial reserves of vitamin A in their livers, were either left entire or were castrated through the kind co-operation of Dr E. Kodicek. Some of the castrated animals received testosterone by injection, others oestradiol, while the rest had no injections. All the animals were kept on a basal diet with 80 i.u. vitamin A daily for about 10 days, and were then killed. The mean concentration of vitamin A in the kidneys was 4.3 i.u./g. in the entire rats, 5.3 in the castrated animals treated with testosterone, 2.9 in those castrated and treated with oestradiol,

and 3.0 in the castrated animals given no injections. Throughout these experiments the kidneys were unusually low in vitamin A for male animals. The results suggest, however, that castration tended to lower the concentration of vitamin A in the kidneys, and that its effects might be neutralized by testosterone but not by oestradiol.

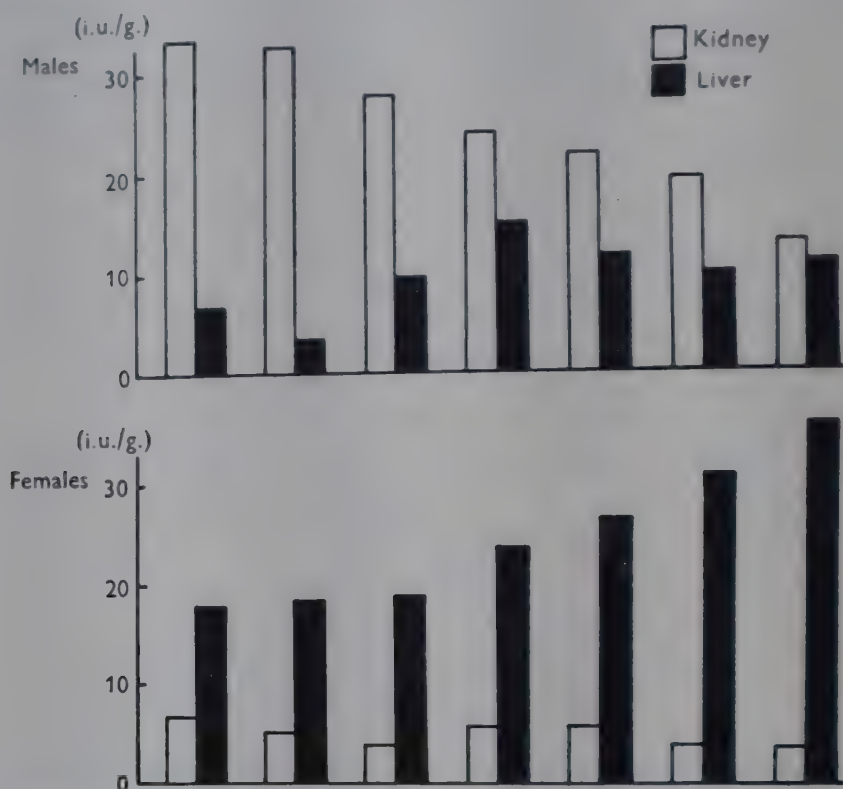


Fig. 3. Concentration of vitamin A in the liver and kidneys of individual male and female rats given 40 i.u. vitamin A daily.

In another experiment depleted female rats were given 40 i.u. vitamin A daily for several weeks in conjunction with a basal diet containing 0.1 % of stilboestrol. The subcutaneous adipose tissues had a bright golden fluorescence. Extraction with ether gave fat with a vitamin A content of 5 i.u./g. The fluorescence, however, remained with the residue of the tissues, from which it could be extracted with hot 50 % ethanol. The adipose tissues of rats not given stilboestrol showed no yellow fluorescence.

Vitamin A reserves in human diseases

Table 2 presents a rearrangement of data collected by Moore (1937) on the median vitamin A reserves found in groups of human subjects who died by accident within 7 days of injury or from various diseases. It will be seen that reserves above the level of those dying from accidents, of 220 i.u./g. liver, were found in diabetes and also in thyroid diseases. In poisoning and hernia the reserves were only slightly below the level for accident cases, but with all other causes of death they were considerably less. It appears, therefore, that in most fatal diseases at least half the vitamin A reserves disappear, although it is uncertain whether the loss is due mainly to increased destruction or is partly due to defective absorption or to a reduced intake of food.

Further examination of the data indicates that the reserves are more severely reduced in some diseases than in others. Thus infections of the head and spine gave a median

Table 2. Median vitamin A reserves in the livers of human subjects, aged 15-59 years, who died from accident or from various diseases (rearranged from Moore, 1937)

Cause of death	No of cases	Vitamin A reserves (i.u./g. liver)	Cause of death	No. of cases	Vitamin A reserves (i.u./g. liver)
Thyroid diseases	9	310	Septic endocarditis	33	90
Diabetes	15	300	Thrombosis, embolism	23	89
Accident	40	220	Bronchiectasis	12	82
Poisoning	13	170	Subacute nephritis	12	75
Hernia and some other conditions	10	160	Peritonitis	12	75
Blood diseases	31	130	Enteritis, colitis	11	74
Cerebral haemorrhage	26	120	Head, spine infections	52	73
Gastric and duodenal ulceration	43	110	Pneumonia	22	63
Appendicitis	19	110	Empyema	12	60
Gall-bladder diseases	13	110	Valvular heart disease	56	60
Cancer	76	110	Abscesses	35	51
Nerve degenerations	13	110	Prostate diseases	23*	40
Coronary disease	20	100	Chronic nephritis	48	25
Tuberculosis	26	96	Urinary infections	13	19
Syphilitic aortitis	27	95			

* All ages.

value of 73 i.u./g. liver and abscesses in other sites one of 51. For pneumonia the median value was 63, and for empyema 60. The lowest values of all were found for urinary diseases, with 25 for chronic nephritis, and 19 for urinary infections. It may be significant that some of the diseases in which low vitamin A reserves were found, including pneumonia, abscesses and urinary infections, are among the more common terminations to vitamin A deficiency in experimental animals. Particular attention may perhaps be directed to the several independent links which now appear to connect urinary diseases with vitamin A deficiency or abnormalities in vitamin A metabolism. Thus it has long been known that vitamin A deficiency in rats frequently leads to fatal urinary infections, often with the production of calculi. In our experience these infections are by far the most common termination to prolonged or repeated vitamin A deficiency. From the more recent investigations described on p. 120 we have learnt that when only small amounts of vitamin A are available the kidney has priority, even over the liver, in absorbing them. In chronic nephritis and urinary infections the vitamin A reserves are usually low, and often completely exhausted. Chronic nephritis is prominent among those diseases in which vitamin A is lost by urinary excretion, and in nephrosis the failure of the liver to absorb vitamin A from the blood plasma has been strongly indicated. Much further research will, however, obviously be necessary before it can be decided how far vitamin A deficiency, either dietary or conditioned in origin, affects the progress of human kidney diseases.

Vitamin A in human blood

Investigations of the levels of vitamin A and of carotenoids in the plasma of inhabitants of this country have been made recently by Moore & Leitner (1949) and by Campbell & Tonks (1949). These results, together with those of the American workers

Kimble (1939), Abels, Gorham, Pack & Rhoads (1941) and Murrill, Horton, Leiberman & Newburgh (1941) are given in Table 3. Some uncertainty in the comparison of the British and American results for vitamin A must arise from unintentional differences in

Table 3. *Values for vitamin A and carotenoids in human blood plasma*

Authority	Country	No of subjects	Carotenoids (i.u./100 ml.)	Vitamin A (i.u./100 ml.)
Moore & Leitner (1949)	Great Britain	195	150	120
Campbell & Tonks (1949)	Great Britain	110	133	108
Kimble (1939)	United States	64	294	109
Abels <i>et al.</i> (1941)	United States	124	325	160
Murrill <i>et al.</i> (1941)	United States	45	343	104

the magnitude of the units (cf. Hume, 1951), but the round figure of 120 i.u./100 ml. quoted by Moore & Leitner is not far removed from the American values. The values for carotenoids, however, appear to be much greater in America than in Britain, probably on account of dietary differences.

As a point of interest it may be mentioned that in collaboration with Dr H. C. Trowell of the Uganda Medical Service we have found that carotenoids are often virtually absent from the plasma of native infants suffering from kwashiorkor. Individual values found were 6, 10, 11 and 25 i.u./100 ml., and ranged from about 2 to 8 % of the values found for healthy, well-nourished Americans. It remains to be decided whether this virtual absence of carotenoids from the plasma is dietary in origin or is a secondary effect of the disease.

Physiological factors affecting the level of vitamin A in the plasma. Our knowledge of the factors which control the mobilization of vitamin A from the liver into the blood plasma is still very limited. According to Clausen and his co-workers, the administration of alcohol to dogs or human beings causes a rise in the vitamin A level of the plasma (Clausen, Baum, McCoord, Rydeen & Breese, 1940; Clausen, Breese, Baum, McCoord & Rydeen, 1941). In the Sheffield experiment, however, heavy doses of alcohol had no effect on the vitamin A level of two of the volunteers who were tested (Hume & Krebs, 1949).

Sex is obviously an important factor in influencing the plasma level. In examinations of human plasma both Kimble (1939) and Moore & Leitner (1949) observed slightly higher mean values for males than for females. In recent experiments with rats we have observed differences in the same direction between groups of males and females given various doses of vitamin A. Using immature pullets, Chapman, Gluck, Common & Maw (1949) have obtained spectacular results with combined injections of oestradiol and testosterone, the vitamin A content of the plasma being about doubled. According to Bodansky, Lewis & Lillienfeld (1943), pregnancy has a slight effect on the plasma level of vitamin A in human subjects, causing decreased values in the later stages.

Effect of disease on the level of vitamin A in the plasma. The level of vitamin A in the plasma may be influenced by disease in various ways. The effect of diseases characterized by the defective absorption of fat has already been mentioned (p. 120). Disease may interfere also with the mechanisms controlling the transfer of the vitamin between the

liver, plasma and other tissues, or may increase the rate of expenditure of the vitamin above the physiological level.

The effects of fever were extensively investigated by Lindqvist (1938), who found greatly reduced levels of vitamin A in the plasma in pneumonia, influenza, tuberculosis and other infections. The levels returned to the normal range promptly on recovery. Many other workers have amply confirmed these observations. It is interesting that Moore (1937) found low liver reserves in fatal cases of pneumonia, suggesting that the decline in the plasma level in this disease indicates not only a failure in mobilization, but the actual loss of a large fraction of the vitamin A reserves of the body.

Shank, Coburn, Moore & Hoagland (1944) have investigated the relationship between vitamin A and rheumatic fever. The low reserves frequently observed by Moore (1937) and Ellison & Moore (1937) in vascular heart diseases suggested that prolonged attacks of this disease might adversely affect the vitamin A status. With Dr Leitner we have made numerous serial estimations of vitamin A in the plasma of patients suffering or recovering from acute rheumatism. In general, the average level of vitamin A rose towards the normal average during the first few weeks of recovery, the return to normal matching the decline in the body temperature and erythrocyte sedimentation-rate. The carotenoid levels, however, remained much below the normal average.

Clear evidence that a very low level of vitamin A in the plasma does not necessarily imply exhaustion of the liver reserves was obtained by Harris & Moore (1947) in a fatal case of infective hepatitis. A specimen of plasma collected a few days before death contained only 19 i.u./100 ml., whereas the liver contained no less than 900 i.u./g., or about three times the average level.

With Dr Leitner we have noticed that the level of vitamin A in the plasma is often very low in patients with fatal illness, even when pyrexia is absent. Thus, estimations on eight patients made within 14 days of death gave a mean value of only 38 i.u./100 ml. The conclusion that fever is not the only factor influencing the ability of the plasma to maintain a normal concentration of vitamin A is supported by the frequent occurrence of very low values in certain skin diseases, which is discussed more fully by Dr Leitner (Leitner, 1951).

Urinary excretion of vitamin A

Since vitamin A is soluble only in fat, its presence would not be expected in urine. This conclusion is fully borne out for normal human urine, which is completely free from the vitamin even when large doses are ingested. Boller & Brunner (1936) and Boller, Brunner & Brodaty (1937), however, made the surprising discovery that patients with certain diseases, including pneumonia, chronic nephritis, and icterus with closure of the biliary duct, passed considerable amounts of vitamin A into their urine. Catel (1938*a, b*) made the no less unexpected observation that large amounts of vitamin A are usually present in dog's urine.

The presence of vitamin A in the urine in pneumonia was confirmed, the amount lost reaching 3000 i.u. daily in one patient (Lawrie, McArdle & Moore, 1938; Lawrie, Moore & Rajagopal, 1941). Smaller amounts were excreted in chronic nephritis. The urine of apparently healthy dogs contained the vitamin, but none was excreted by rats,

even when diseased, or by rabbits. Vitamin A in human urine was not accompanied by carotenoids, as in the plasma. Patients excreting the vitamin always had some degree of proteinuria, but heavy excretion of protein was sometimes observed without any excretion of the vitamin.

Some outstanding problems

The numerous investigations that have been reviewed admittedly represent only a small fraction of the material which is available. They indicate clearly, however, that the vitamin A levels in the body may be influenced by many factors other than the dietary intake, and that lesions which could be caused by vitamin A deficiency are a commonplace in many diseases in which the sufferers are receiving ordinary diets.

In further research more information should be sought on the factors controlling the mobilization and distribution of the vitamin. Can secondary deficiency result through failure in the mobilization mechanism even when vitamin A is present in the liver? If so, are there any means available to rectify faulty mobilization? Another problem is presented by the disappearance from the body of large amounts of the vitamin, under either normal or pathological conditions. What are the degradation products? In the behaviour of retinene and of vitamin A acid we have hints that the oxidation products of the vitamin may have biological importance, but we remain ignorant as to its ultimate metabolic fate.

Vitamin A and metabolic stress. Hardly less important is the question of whether any benefit, beyond provision for the future, is derived from an intake of vitamin A substantially above the minimum level, such as allows the accumulation of a substantial liver reserve. Have the large amounts of vitamin A which we know to be present in the livers of most of the inhabitants of this country any functional purpose?

An important step towards answering this question has recently been made by Meunier, Ferrando, Jouanneteau & Thomas (1949). When rats were given a diet deficient in vitamin A and containing 2 % of sodium benzoate, they declined and died, even when given a dose of 2.5 μ g. vitamin A, which would amply have supplied their requirements in the absence of sodium benzoate. Good growth and survival for an indefinite period were, however, secured even in the presence of benzoate by increasing the vitamin A allowance to 20 μ g. daily. A substance which was toxic at the lower level of dosing with vitamin A thus became innocuous at the higher level. Further experiments, made later with bromobenzene, gave similar results (Meunier, Ferrando & Perrot-Thomas, 1950).

In investigations along similar lines we have examined the effect of the vitamin A allowance on the resistance of rats to stilboestrol poisoning. Both vitamin A deficiency and stilboestrol cause cornified vaginal smears in rats, and it seemed of interest to oppose the vitamin with its curative action on deficient animals to an agent which would not allow the vaginal surface to function normally. With doses of 4 i.u. vitamin A daily, the presence of stilboestrol in the diet reduced the mean weight increase in 4 weeks to 6 g. from 23 g., whereas with a daily dose of 40 i.u. the weight increase was reduced only to 25 g. from 34 g.

In other experiments we have obtained indications that the adverse effect of an unheated room on the growth of rats may sometimes be partially corrected by increasing the vitamin A allowance. The conditions necessary to demonstrate this possible beneficial action of the vitamin were, however, achieved in two only out of four experiments.

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Pathology of Vitamin A Deficiency and its Clinical Significance

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Pathology is concerned with the response of living organs or tissues to injury. If the injury is not lethal repair or gradual adaptation follows. Deficiency of a single vitamin rarely occurs naturally but, as a conditioned deficiency, may provoke considerable damage to the living cells (Leitner, 1948). The morphological changes in such a conditioned deficiency may reflect the peculiar biochemical damage caused to one type of cell in one single organ, or to the same type of cell distributed in different tissues; in the latter case, secondary effects such as loss of function and inanition may affect the whole organism.

Tissue changes in vitamin A deficiency

For lack of space, only certain topics can be considered.

Epithelial tissue. The effects of vitamin A deficiency on the epithelial tissue may be summarized as atrophy of the epithelial layer followed by reparative proliferation of the basal cells with growth and differentiation into a stratified keratinizing epithelium (Wolbach & Howe, 1925, 1926). These changes occur in man and presumably in all vertebrates, even in the foetus (Wilson & Warkany, 1947). Mainly affected are epithelia with secretory function, such as the salivary glands, including the tongue and pharynx, the respiratory tract with the trachea and bronchi, the eyes including the cornea, conjunctivas and intra- and extra-orbital glands, the genito-urinary tract with the renal pelvis, ureters and bladder, and the sex glands. In man, the skin also may be involved. Keratinization in the secretory cells may produce severe obstruction in the gland ducts with cystic dilatation and accumulation of debris leading to severe infection of the surrounding tissues (Wolbach & Bessey, 1942) (Pls. 1 and 2). On the other hand, epithelial cells with chemical function and capable of division, such as those of the liver, do not show marked atrophy, and do not exhibit keratinizing metaplasia.

After vitamin A administration, each individual epithelium reassumes its normal structure and function, when the keratinized cells have been removed by autolysis and leucocytic infiltration (Wolbach & Howe, 1933 *a, b*).

Incisor teeth of rodents. In rats and guinea-pigs the enamel organ, which is of epithelial origin, atrophies and undergoes keratinizing metaplasia in vitamin A deficiency. The odontoblasts which are cells of mesenchymal origin are then unable to deposit dentin in the normal way and produce an abnormally thick layer on the labial side, and an abnormally thin one elsewhere. After vitamin A administration regeneration of the enamel organ with resumption of normal dentin formation takes place (Wolbach & Howe, 1933 *a, b*; H. Mellanby, 1939). Boyle (1933) described similar changes in a human infant, and hyperplastic keratinized gums have been observed in man (King, 1940).

Skeleton. If vitamin A deficiency is established at a sufficiently early stage, skeletal growth in rats may be retarded in a way involving only the endochondral bone formation. On the other hand, there is an excess of periosteal bone formation, whilst the soft tissues, including nerve tissue, continue to grow until general inanition occurs. The

degeneration of nerve tissue in vitamin A deficiency was first noticed by Mellanby (1931, 1935). He found later that the experimental dogs became deaf, and proved that the deafness was due mainly to bony overgrowth of the labyrinth and only secondarily to nerve change (Mellanby, 1938). It was further suggested (Mellanby, 1939*a, b*; Wolbach & Bessey, 1940, 1941) that all nerve lesions due to vitamin A deprivation are caused by bone pressure; thus, overcrowding of the cranial cavity causes multiple herniation of the cerebrum and cerebellum into the dural sinuses; overcrowding of the spinal cord causes herniations of the nerve roots into the intervertebral foramina, and pressure leads to degeneration of the nerve roots, peripheral nerves and various cerebral tracts. Mellanby (1939*a, b*) found compression of the optic nerve and signs of raised intrathecal pressure due to thickening of the cranial bones. Subsequently, he demonstrated compression of the olfactory, optic, trigeminal and cochlear nerves and also of the pituitary body in puppies and rabbits (Mellanby, 1941, 1943*a, b*). After vitamin A administration the changes are to some extent reversible (Mellanby, 1947). Such nerve lesions caused by bone pressure have not yet been recognized as an effect of vitamin A deficiency in man, but are quite common in cattle under field conditions; in calves, for instance, narrowing of the optic foramen constricts the optic nerve, and causes papilloedema and blindness (L. A. Moore, 1939); increased cerebrospinal fluid pressure also (L. A. Moore & Sykes, 1940) has often been observed.

Nerve tissue. Whether degeneration of nerve tissue occurs as a direct result of vitamin A deficiency has been a controversial issue during the last 20 years (Mellanby, 1931). Several authors have produced valid evidence of the occurrence of direct nerve-tissue lesions (Rao, 1940; Irving & Richards, 1940), and recently further evidence has been presented that bone pressure is not the only explanation. Ducks were maintained on a vitamin A-deficient diet and after from 10 to 15 days showed muscular weakness, incoordination, ataxia and paralysis. On histological examination, there was sliding of the grey and white matter over one another, with haemorrhage, necrosis, degeneration of nerve cells and tracts, and intermedullary formation of cartilage and bone (Fletcher & Rigdon, 1949). Such changes, especially the finding of cartilage and bone within the nerve substance, cannot easily be related to the mechanical theory, as the makers of these observations point out.

Congenital abnormalities related to vitamin A deficiency

Results of animal experiments provide convincing evidence that nutritional deficiency in pregnancy may not produce any signs in the mother but that the young may die during the first few days of life. Another important concept is that the frequency of occurrence of a hereditary congenital malformation may be enhanced by dietary deficiency in pregnancy, so that the latent tendency to a congenital trait, only rarely manifest in favourable conditions, may be expressed if the antenatal diet is deficient, especially in vitamin A. The conclusion is a difficult one to apply to man, but it can be reasonably assumed that neonatal mortality and incidence of congenital malformations increase in unfavourable nutritional circumstances.

In the offspring of pigs given a diet deficient in vitamin A, Hale (1933, 1935, 1937)

found blindness with anophthalmos or microphthalmos, accessory ears, cleft lip and palate, and misplaced kidneys. More systematic investigations by Warkany and his colleagues amplified the results (Warkany & Schraffenberger, 1944, 1946; Warkany & Roth, 1948; Warkany, 1948; Wilson & Barch, 1949). They found that the fertility of rats kept on a vitamin A-poor diet was low; some resorbed their young; the offspring of others were often stillborn or died within a day or two. If they survived, their size was less than normal, and they were oedematous and frequently had subcutaneous haemorrhages. In about 75 %, ocular abnormalities were present, such as defective eyelids, a fibrous mass behind the lens in place of the vitreous body, coloboma or eversion of the retina, and rudimentary development of the iris, ciliary body or anterior chamber. Other congenital defects regularly produced were in the development of the lungs and pleural cavity, in the aortic arches and in the interventricular septum; other defects were spongy myocardium, diaphragmatic hernias (Wilson & Warkany, 1949, 1950), undeveloped renal pelvis and ureters, fused and horseshoe kidneys, undescended testes, absence of seminal vesicles and bulbo-urethral glands, and complete lack of vagina (Wilson & Warkany, 1948). The difficulty of applying the results of these animal experiments to man was pointed out by Jackson & Kinsey (1946) who confirmed Warkany's work on eye defects. The commonest ocular malformation in rats was a kind of retrolental fibroplasia, a disease which in recent years caused blindness in about 8 % of premature babies under 5 lb. in the United States. The intake of vitamin A required to produce this malformation in rats had, however, to be less than 16 % of the normal, a degree of deficiency unlikely to occur in pregnant women. In contrast, however, evidence has been presented quite recently of a possible correlation between the incidence of retrolental fibroplasia and the use of large amounts of iron and water-miscible vitamins in man; it is claimed now that large doses of vitamin E reduce the incidence of this disease (Owens & Owens, 1949).

Fibrocystic disease of the pancreas. It is of interest to make reference here to two inherited diseases encountered in man, for which some relationship to vitamin A deficiency is claimed. Fibrocystic disease of the pancreas (Andersen, 1938; Blackfan & May, 1938) was diagnosed, before 1938, mainly after death, and appears invariably to lead to death before the age of 16 (Parmelee, 1935; Mattheson, 1949). The condition is characterized by steatorrhoea, a low level of vitamin A in the blood, and epithelial changes like keratinizing metaplasia in the lungs and pancreas and occasionally in other organs. Apart from the isolated cases admitted to hospital, usually during the first few days after birth, with the diagnosis of meconium ileus for want of pancreatic digestion (Farber, 1944), the large majority of the infant sufferers perish from recurrent bronchopneumonia. Reviewing the circumstances in twenty families affected by the disease, Andersen & Hodges (1946) suggested that the condition might be due to an inherited recessive trait, or even to an incomplete dominant (Cockayne, 1947). Andersen (1949a-c) considers that the condition is of nutritional origin, and she maintains that in cases diagnosed at an early stage dietary therapy, including large doses of vitamin A, prevented the development of chronic respiratory infection. The theory of a nutritional aetiology has been contested recently and the view has been advanced that the vitamin A changes are only of a secondary nature (Bodian, 1950).

Pityriasis rubra pilaris. Another disease characterized by a low level of vitamin A in the blood and generalized keratinizing metaplasia is pityriasis rubra pilaris which will be discussed later in more detail. The existence of two different forms, a hereditary and an acquired one, has been accepted until recently (Touraine, 1942). After having collected 152 cases from the world literature, the present author was able to demonstrate with the help of Ford that pityriasis rubra pilaris is always inherited, probably as a simple, autosomal, heterozygous condition, without being even partially sex-controlled, and without linkage with the blood group (Leitner, 1947*a*). The apparently non-familial cases are due to mutation (Leitner & Ford, 1947; Cockayne, 1950).

Only two patients with pityriasis rubra pilaris have been encountered, in connexion with whom, in spite of thorough search, no other affected member of the family could be traced. One was a man affected first at the age of 63; one of his children was in every way normal, but the other, a well-nourished and healthy woman of 30, had the pathologically low level for vitamin A in the plasma of 44 i.u./100 ml. (Barber & Leitner, 1951). Skipping of a generation and reduced expressivity of a gene have been occasionally observed in families even with dominant abnormal traits, but microforms of the abnormality or slight deviations from the normal may often be detected only by special examination.

The second patient had syringomyelia as well as pityriasis rubra pilaris (Barber & Tatz, 1946). The great importance of this case lies in the fact that both conditions were inherited, and that two different systems of ectodermal origin, the skin and the central nervous system, were involved in the same patient (Leitner, 1946).

Clinical significance of vitamin A deficiency

Confronted with the abundant literature of the past 20 years on the manifold symptoms of vitamin A deficiency, and with the predominantly negative results of the Sheffield experiment on young, healthy conscientious objectors (Hume & Krebs, 1949), one is hesitant to embark on this chapter. Clinical medicine, however, in contrast with the Sheffield and other experiments (Steffens, Bair & Sheard 1939), deals with subjects of every age group, and with known or unknown impairments of the organs, who in addition are exposed to a number of conditioning factors (Leitner, 1948). Diseases caused by lack of water-soluble vitamins are easily detectable, but it is much more difficult to relate symptoms and signs to a lack of vitamin A, of which reserves may be stored in the organs for up to 2 years. For this reason the discussion of the clinical aspect should be considered, not so much as a statement of facts, but rather as an assessment of views prevailing at present.

It is quite impossible to deal adequately with the whole clinical significance of vitamin A deficiency in the available space. It may, however, be briefly mentioned that the antagonistic action of vitamin A and thyroxine (Fasold & Peters, 1933), postulated for many years, has been confirmed (Sadhu, 1948). In two patients suffering from thyrotoxicosis, clinical cure was achieved by daily peroral doses of 200,000–400,000 i.u. vitamin A after 4–8 weeks (Simkins, 1947). Continental workers have claimed good results in treating several clinical conditions, including pulmonary tuberculosis (Lafontaine, 1948) and hypertension (Bonfils, 1947).

Relation of vitamin A to the health of the eye. The changes which take place in the eye when the organism is deprived of vitamin A are too well known to need description. The first change causes hemeralopia, and later Bitot's spots, xerophthalmia and keratomalacia appear.

Relation of vitamin A to cutaneous diseases. Keratinizing metaplasia has already been mentioned as the cardinal change in vitamin A deficiency, a view that has been accepted by paediatricians (Blackfan & Wolbach, 1933; Sweet & K'ang, 1935) and by nearly all workers interested in nutrition. There are, however, difficulties in its application to cutaneous diseases in man.

An interesting point is the fact that, even after excessive amounts of vitamin A had been given, it could not be detected by fluorescence microscopy in the epidermis (Cornbleet & Popper, 1942), or epithelium of mucous membranes, which are considered to be the first sites of vitamin A deficiency (Popper, 1941). Moreover, conservative pathologists still maintain that keratinizing metaplasia is caused only by irritation or chronic inflammation, though Beattie, Dickson & Drennan (1948) state that metaplasia may result from impairment of nutrition and function.

In experiments on rats, no true keratinizing metaplasia could be produced by irritation or by giving oestrogens, unless the animals were deprived of vitamin A (McCullough & Dalldorf, 1937). The opposite was, however, never conclusively proved, namely that the same type of metaplasia cannot be achieved by any other means in adequately fed rats. A varying degree of hyperkeratosis, comparable with that seen in man, the extent of which was inversely related to the amount of vitamin A ingested, was produced in rats by Moulton (1943). No conclusive evidence for the experimental production of follicular keratosis in man has, however, been presented as yet, even when the deprivation has been maintained for as long as 25 months (Hume & Krebs, 1949).

The extensive literature on human skin lesions attributed to vitamin A deficiency has been repeatedly discussed recently (Stannus, 1945; Leitner 1945, 1947*a, b*; Leitner & Moore, 1946*a, b*; Marrack, 1948). Though we have no knowledge about its mechanism, there seems little doubt that vitamin A is one of the main factors essential for the normal metabolism of the epithelium. Little is understood of its interrelationship with such other factors as the vitamin B complex (Sullivan & Evans, 1945), traumata (Stannus, 1945), or vitamin C deprivation, which also may cause follicular hyperkeratosis (Wiltshire, 1919). Moreover, vitamin A is usually administered in oily solution containing unsaturated 'essential' fatty acids (linoleic, arachidonic, linolenic) which also are claimed to be necessary for the maintenance of the normal skin (Hansen, 1937; Burr & Barnes, 1943).

If vitamin A deficiency is the primary cause of follicular keratotic and other cutaneous conditions, like Darier's disease, pityriasis rubra pilaris, and ichthyosis, cure should be effected by administration of average doses of vitamin A. The fact that well over a hundred times the minimum daily dose of 1250 i.u. is needed, indicates that vitamin A deficiency cannot be the primary cause. The therapeutic effect conferred is not necessarily specifically nutritional; such doses may act by correcting hypochlorhydria (Földes & Vajda, 1941), by influencing the allergic state (Obermeyer & Frost, 1945),

or by ‘supercharging’ (Leitner & Moore, 1946*a*). In the last instance the excess of vitamin A which cannot be retained and stored in the liver may appear in tissues from which it was excluded by the disease, where it may restore an equilibrium previously upset, and may control the regeneration of epithelial tissues.

Many other cutaneous conditions have been associated with vitamin A deficiency; such are phrynoderma (Nicholls, 1933), acne vulgaris (Straumfjord, 1943; Davidson & Sobel, 1949), ichthyosis (Rapaport, Herman & Lehman, 1942), leukoplakia vulvae (Hyams & Bloom, 1947) and pili torti (Siskind, 1947), and therapeutic successes have been reported.

Some recollections of our experiences with Darier’s disease and with pityriasis rubra pilaris (Leitner, 1947*a*; Leitner & Moore, 1946*a, b*, 1948) may be of interest. Both diseases are characterized by hyperkeratosis in relation to the pilosebaceous glands and hair follicles, and the lesions are similar to those which were originally found in association with vitamin A deficiency (Frazier & Hu, 1931; Loewenthal, 1933; Nicholls, 1933, 1934). Table 1 shows the mean plasma content of carotenoids and vitamin A for

Table 1. *Mean values for carotenoids and vitamin A in the plasma of 105 persons with common skin diseases*

Disease group	No. of cases	Carotenoids (i.u./100 ml.)	Vitamin A (i.u./100 ml.)
Eczemas	26	109	126
Pityriasis rosea	5	119	92
Psoriasis	10	151	117
Seborrhoea	11	144	128
Lupus vulgaris	4	214	84
Lichenifications	13	139	123
Acne vulgaris	4	99	119
Rosacea	8	100	122
Skin infections	11	132	119
Alopecia	5	186	114
Pruritus	3	139	128
Verruca	1	84	101
Urticaria pigmentosa	1	55	78
Erythema nodosum	1	170	127
Lymphogranuloma inguinale	1	106	73
Pityriasis lichenoides chronica	1	104	284
Mean for all cases		130	120

105 hospital cases suffering from a variety of skin diseases; Table 2 gives the same values for 116 healthy subjects and hospital patients suffering from conditions not likely to alter the vitamin A level. The figures are almost identical. Table 3 shows the values in nine cases of Darier’s disease, Table 4 in eight cases of pityriasis rubra pilaris and Table 5 in seven cases of ichthyosis. It is quite evident that in all three conditions both the carotene and vitamin A values are definitely below normal though, like others, we found in some cases quite normal levels (Peck, Chargin & Sobotka, 1941; Peck, Glick, Sobotka & Chargin, 1943; Carleton & Steven, 1943; Weiner & Lewin, 1943; Porter, Brunauer & Godding, 1947). It may be said, however, that in our material, with a few exceptions, the initial vitamin A values corresponded roughly to the degree of clinical involvement. The initial vitamin A values in our patients with

Darier's disease were lower than in those with pityriasis rubra pilaris, but after dosing the highest vitamin A values were much higher and more quickly attained in Darier's disease than in pityriasis rubra pilaris. A constant high vitamin A level is, however, no guarantee of the success of treatment. For example, case no. 5 of Darier's disease

Table 2. *Mean values for carotenoids and vitamin A in the plasma of 116 healthy subjects and hospital patients with diseases unlikely to affect the plasma value for vitamin A*

Group	No of cases	Carotenoids (i.u./100 ml.)	Vitamin A (i.u./100 ml.)
'Normal'	41	161	118
Accidents	4	141	78
Fractures	12	102	92
Arthritis	18	145	114
Varicose veins	19	110	123
Hernias	18	133	110
Haemorrhoids	4	139	117
Mean for all cases		139	113

Table 3. *Mean values for carotenoids before dosing with vitamin A, and of vitamin A before and after dosing with vitamin A, in the plasma of nine patients with Darier's disease (keratosis follicularis)*

Case no.	Age (years)	Sex	Vitamin A				
			Carotenoids, before dosing		Before dosing		After dosing
			Mean value (i.u./100 ml.)	Lowest value (i.u./100 ml.)	Mean value (i.u./100 ml.)	Lowest value (i.u./100 ml.)	Highest value (i.u./100 ml.)
1	13	M.	102	100	78	55	1276
2	33	F.	171	59	92	55	268
3	61	F.	215	199	57	20	687
4	31	M.	90	57	57	7	790
5	53	F.	62	61	55	28	1846
6	19	F.	113	95	106	103	278
7	20	F.	45	37	70	45	—
8	52	M.	94	85	131	116	—
9	34	F.	153	153	66	66	—
Mean			116	94	79	55	857

(Table 3) had very high values for many years, but the improvement of the skin condition was slight. Case no. 8 of pityriasis rubra pilaris (Table 4) never attained vitamin A values above 227 i.u./100 ml., but the very serious condition healed clinically after 4 months' treatment (Barber & Leitner, 1951). We are still of the same opinion as before that 'this does not imply that these conditions are solely due to any A-avitaminosis nor that vitamin A can cure these diseases' (Leitner, 1945).

Relation of vitamin A to hepatic diseases. Since Moore (1929) first demonstrated that rats fed on carotene stored vitamin A in the liver, it has been repeatedly shown that liver diseases may interfere with vitamin A metabolism, but keratomalacia (Thompson, 1894) and xerosis (Bloch, 1924*a, b*) were described much earlier in jaundiced children. In liver diseases not only does the absorption of vitamin A appear to be impaired (Breese & McCoord, 1940) but its release from the liver is disturbed also (Meyer, Steigman, Popper & Walter, 1943; Popper, Steigman, Meyer & Zevin, 1943). Though

about 95 % of the vitamin A stored is stored in the liver (Moore, 1931), the vitamin A level in the plasma in infective hepatitis is low in spite of adequate liver stores (Harris & Moore, 1947). In long-standing and extensive liver lesions, as in liver cirrhosis, not only the liver reserves (Wolf, 1932; Moore, 1937), but also the plasma vitamin A levels, are reduced (Haig & Patek, 1942). Vitamin A tolerance curves indicate that the level of

Table 4. Mean values for carotenoids before dosing with vitamin A, and for vitamin A before and after dosing with vitamin A, in the plasma of eight patients with pityriasis rubra pilaris

Case no.	Age (years)	Sex	Vitamin A				
			Carotenoids, before dosing		Before dosing		After dosing
			Mean value (i.u./100 ml.)	Lowest value (i.u./100 ml.)	Mean value (i.u./100 ml.)	Lowest value (i.u./100 ml.)	Highest value (i.u./100 ml.)
1	14	M.	41	31	48	46	408
2	41	F.	158	110	145	66	690
3	2·5	F.	43	42	44	35	140
4	46	M.	93	28	90	80	—
5	12	F.	200	200	140	85	—
6	51	M.	105	102	97	80	—
7	21	F.	151	93	96	70	255
8	63	M.	85	84	34	32	227
Mean			109	86	86	61	344

Table 5. Mean values for carotenoids, and for vitamin A, in the plasma of seven patients with ichthyosis, before dosing with vitamin A

Case no.	Age (years)	Sex	Carotenoids		Vitamin A	
			Mean value (i.u./100 ml.)	Lowest value (i.u./100 ml.)	Mean value (i.u./100 ml.)	Lowest value (i.u./100 ml.)
1	28	M.	98	77	86	80
2	13	F.	72	65	72	49
3	32	F.	145	145	55	55
4	30	F.	61	61	50	50
5	8	F.	33	33	19	19
6	31	M.	178	169	114	104
7	40	M.	115	112	115	114
Mean			100	94	73	67

vitamin A in the blood is related to the degree of liver damage (Ralli, Papper, Paley & Bauman, 1941; Popper, Steigman, Meyer & Zevin, 1943; Popper, Steigman & Zevin, 1943). There seems also to be a rough parallelism between the extent of the liver damage, as indicated by the hippuric-acid tolerance test, and the magnitude of the vitamin A values in the blood (Harris & Moore, 1947). This may explain why, in Darier's disease and pityriasis rubra pilaris, we found in some cases severe impairment of liver function particularly when we were using the hippuric-acid test (Leitner, 1947a; Leitner & Moore, 1946b, 1948), whereas Porter & Brunauer (1949), using mainly other liver function tests, could find only minor abnormalities.

The urinary excretion of vitamin A in kidney and other diseases may be connected with the inability of the damaged liver to store the vitamin.

Relation of vitamin A to febrile states. Paediatricians were, for many years, aware of the fact that very low plasma vitamin A levels were recorded in febrile diseases, especially in pneumonia (Lindqvist, 1937; Clausen & McCoord, 1938). Analysing the livers of children who had died of pneumonia, Lindqvist (1938) found adequate liver stores, although the vitamin A level in the blood had been found very low a few days previously. In convalescence after feverish diseases vitamin A levels much higher than before the onset of the disease have repeatedly been recorded (Josephs, 1943; Steigman, Meyer & Popper, 1945; Harris & Moore, 1947).

The question arises whether the infective process, as such, or the elevation of the body temperature was responsible. In ninety-two patients at the Chicago Intensive Treatment Centre, where syphilis and gonorrhoea are treated by physically induced hyperthermia combined with chemotherapy, elevation of body temperature (rectal) up to 105.5° or 106° F. was followed by depression of the plasma content of vitamin A and of carotene. The diminution of both values was directly related to the duration of fever, being about 10 % after 3 hr. of fever, 20 % after 6, and 30 % after 8. On the 2nd morning after the fever treatment a definite rise in the plasma value for vitamin A was recorded, and on the 3rd morning it returned to the original level without any medication or dietary supplements (Aron, Craig, Farmer, Kendell & Schwemlein, 1946). In acute rheumatism a considerable and persistent fall of the vitamin A level was found, the degree varying directly with the intensity of the rheumatic attack (Shank, Coburn, Moore & Hoagland, 1944).

We approached the question on a somewhat wider basis (Jacobs, Leitner, Moore & Sharman, 1951). Our material consisted of over 300 patients in hospital, including 118 suffering from rheumatic fever, about another hundred with various pyrexial diseases such as pneumonia, pleurisy and tonsillitis, and about hundred more with miscellaneous illnesses. The number of male and female patients was equal. The age of the rheumatic subjects ranged from 5 to 60 years, with an average of 20.5 years, 64 % of the patients being between 5 and 19 years. The other groups included fewer children and more middle-aged subjects. The vitamin A values were lower with increasing body temperature to about the same extent in rheumatic fever, pneumonia, pleurisy, tonsillitis and erythema nodosum. The inverse relation was not, however, absolutely constant.

The claim has recently been advanced that there is a sudden and considerable increase of the circulating plasma volume in rheumatic fever (Reid, Watson & Sproull, 1950). Bradley (1951) attributes the sudden anaemia and heart failure in the acute rheumatic attack to the accompanying decrease in haemoconcentration. This aspect is very interesting, but it is difficult to correlate our results as yet with the very few estimations of blood volume in rheumatic fever published so far. It appears also that the fall of the vitamin A level in our patients was not in proportion to the blood dilution, and it was greater than in experimentally produced fever. In the present state of our experience I am, therefore, inclined to attribute the fall in plasma vitamin A level to disturbances in vitamin A metabolism. The derangement might differ little at first in the different pyrexial states, but in rheumatic fever additional factors come into play, such as the prolonged course of the illness and the frequent relapses at an age when the normal requirements for growth and development are greatest. The end effect of



1



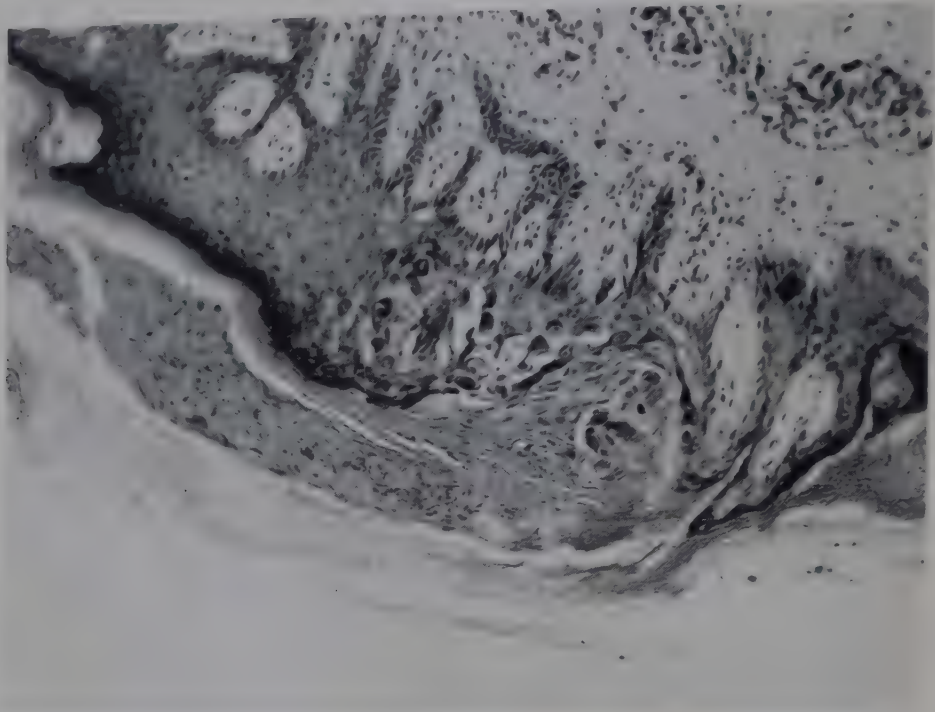
2



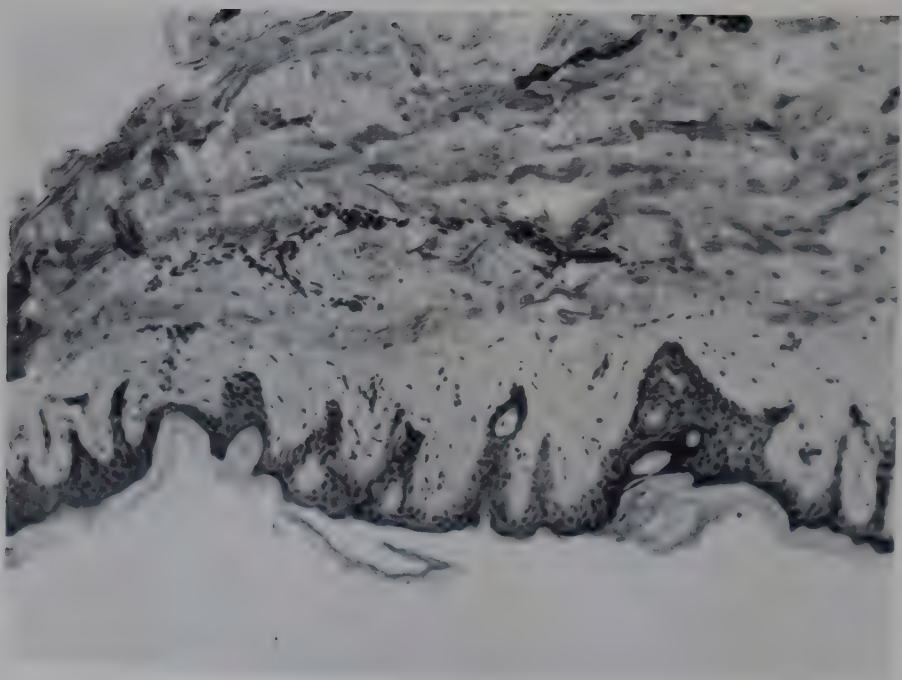
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4



1



2

vitamin A depletion in acute rheumatism may, therefore, appear to be different and more complex. In previous preliminary experiments on rheumatic patients, I had the impression that besides a nutritious diet, a daily supplement of 40,000–50,000 i.u. vitamin A was beneficial (Thornton, 1946).

In conclusion, there are two conditions in which I should like to make a suggestion that seems justified by the present state of vitamin A research, though the therapeutic necessity cannot yet be fully substantiated. I would like to advocate that a daily dose of 40,000–50,000 i.u. vitamin A be given in rheumatic fever, and in pregnancy complicated by febrile states, especially during the first trimester, which corresponds mainly to the organogenic period. Future research may support and possibly widen the range of these, as yet tentative, suggestions.

SUMMARY

1. After a short survey of the principal tissue changes, the results of experimentally produced congenital abnormalities related to vitamin A deficiency have been presented. The possible relationship of vitamin A deficiency to two inherited diseases in man, fibro-cystic disease of the pancreas and pityriasis rubra pilaris, has been discussed.

2. The importance of environmental factors in the manifestation of a latent hereditary trait has been emphasized. It has been suggested on the basis of experimental results that the frequency of a congenital malformation may be enhanced by deficiencies in the diet during pregnancy and, further, that neonatal mortality and the incidence of congenital malformations in man may increase under unfavourable nutritional circumstances.

3. The clinical significance of vitamin A deficiency has been referred to in relation to the ocular system (hemeralopia, Bitot's spots, xerophthalmia, keratomalacia), and has been discussed at greater length in relation to certain cutaneous diseases (Darier's disease, pityriasis rubra pilaris), to involvement of the liver, and to febrile states.

4. The suggestion has been put forward, that it might prove advantageous to give 40,000–50,000 i.u. vitamin A daily in rheumatic fever, and in pregnancy complicated by febrile states, especially during the first trimester.

EXPLANATION OF PLATES

PLATE 1

1. Gland duct filled with keratotic plugs.
2. Gross follicular hyperkeratosis in vitamin A deficiency.
3. Darier's disease before vitamin A treatment.
4. Darier's disease after vitamin A treatment.

PLATE 2

1. Microscopic changes in Darier's disease before vitamin A treatment.
2. Microscopic changes in Darier's disease after vitamin A treatment.

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SIXTY-THIRD SCIENTIFIC MEETING—TWENTY-NINTH SCOTTISH MEETING

USHER INSTITUTE, EDINBURGH

14 OCTOBER 1950

GROWTH

Chairman: PROFESSOR F. A. E. CREW, F.R.S., *Usher Institute of
Public Health, Edinburgh*

Growth and Health

By I. LEITCH, *Commonwealth Bureau of Animal Nutrition, Bucksburn, Aberdeenshire*

A general introduction to a discussion on growth and health might take either of two main forms. It might review what we know about the material requirements for growth: the total energy needs, the grams of protein required to give this or that amount of soft tissue growth, the grams of calcium and phosphorus required for growth of the skeleton; it might discuss the functional importance of substances that make little material contribution to growth, and finally, sum up what we know about the interrelation of diet and resistance to infection. These are the subjects with the details of which most of the observational and experimental work in nutrition is, at the moment, concerned. It would take days to sum up the information; and, when that had been done, I doubt whether we should be able to do more than reaffirm general principles. These general principles are already well known. The concept of a balanced diet has become part of our 'common knowledge'.

Perhaps partly because I shirked that major task, and partly because I think it is good, now and then, to lay aside the microscope and look at things with the naked eye, I have chosen the second approach. In very general terms I am going to ask what we mean by good growth and by health and what the one has to do with the other.

In experimental work with animals most often weight increase is taken as if it were synonymous with growth; less often growth is defined as increase in one or more linear dimensions. Obviously increase in weight may be increase in bone or muscle or fat

or merely water or some combination of any or all of these; and some linear dimensions may be just as difficult to interpret. But suppose we had some complicated technique combining weights and measures and isotope studies and specific gravity of the body, what would it tell us about growth? It would be no more than a means to a crude chemical analysis without killing the subject, and, by itself, it would be just as interesting or as sterile as any other chemical analysis.

Before we can get up any real interest in the result we must know what changes in the structure and composition of the body mean in terms of some standard of value. What is value in growth? Why do we say this animal or person is well grown and that is not? We mean a great deal more, I think, than merely that the one is bigger or heavier than the other. The term well grown and the attitude of mind behind it imply some target of performance which we consider to have been approximated or missed.

Nature and nurture

In an attempt to clarify that idea, I have built up an argument in picture form. In the first place we must go back to the basis of growth, which depends on nature and nurture, but we should not require either complex genetical concepts or the refinements of chemistry to make the argument plain.

The fundamentals of genetics, or all of them that need concern us here, have been known for thousands of years; they are part of everyday language, everywhere. 'Like begets like'; 'like father, like son'; 'what is bred in the bone . . .'; 'you cannot make a silk purse out of a sow's ear': these and similar clichés exist in all languages, sometimes in quaint forms, but with the same meaning. Even primitive man must have recognized the sometimes astonishing resemblance of child to parent, and even so-called primitive races of mankind have long practised systematic selection and breeding of their domestic stock. It is equally true that there has long been an awareness of merit in human reproduction. The ancient Greeks, and even earlier civilizations, had clear views on eugenics; and the same awareness, the same urge to 'good breeding' certainly persists in full force, and sometimes in strange forms, at the present day. I do not know whether, or to what extent, Plato's plans for human stud farms materialized, but modern and better informed people accept similar ideas.

We have no galaxy of clichés about nurture, which is not difficult to understand. It is still impossible to disentangle the effects of different components of the environment, especially in human populations where they can seldom be varied or controlled independently, and where cause and effect are so liable to be confused. For instance, tailors were, at one time, perhaps still are, accepted as of poor physique. But that is not because tailoring interferes with growth that is, in any case, already past, but because the relatively unfit gravitate to occupations that require relatively little physical strength. But better and worse diets were certainly distinguished and are, to some extent, embodied in racial beliefs of one kind and another. Perhaps the attribution of the peculiar virtues of the English to the eating of roast beef may enjoy a scientific rebirth with the ascent of the 'animal protein factor' to its present prominence in experimental work.

The effect of nurture on the concrete and visible expression of nature has, in the

past, received too little attention at the hands of geneticists and plant and animal breeders. Let us review briefly what nurture can do to inhibit the expression of nature and what the significance of that inhibition may be.

In plants. Baur (1911) has described two dandelion plants, one grown high in the Alps on poor soil and in the cold, the other in the warm and fertile plain. If the dandelion were an important food plant, the verdict would be that the big, succulent plant is the better. But consider a different plant. Exactly the same occurs if edelweiss is transported to a warm and fertile garden. It loses its grey colour and white hairs; it stretches out and becomes green and leafy, the flower looks different; in fact an uninteresting plant. The Alpine is the plant that is prized. This little story has two morals. The first is that if one has seen a plant, or animal, only in one environment, all one knows about its genetic constitution is what it can do in that environment and not what it may do or not do in any other. The second is that the terms good and bad, better and worse, as descriptions of plants or animals, are not absolutes, but are valid only in relation to environment, and that they imply some criterion of value which may be utilitarian or aesthetic or both. This is now, of course, well recognized in relation to crop production. For instance, a very large chapter in the history of Canadian agriculture is the story of the search for varieties of wheat that would survive the dry, cold climate of the prairies, that would grow and ripen in the short growing season, and that would resist indigenous diseases.

In animals. This is not yet so well realized in regard to animals, so let us look at what the wrong nurture can do to animal nature.

McMeekan's (1940*a-c*, 1941) papers on the growth of pigs show (Pl. 1, Fig. 1) how a well-bred or 'improved' pig grows. One can see the change in shape as the animal grows from birth to the approved 200 lb. bacon pig. In shape, the original wild pig at the fully grown stage is much more like the improved pig at birth than at maturity. The important thing is that the characteristics that are valued by the pig farmer, who is going to sell pork or bacon, are those which develop last. The most prized parts of the pig are the loin and hind-quarters. And the quality of the joints depends on the distribution of lean and fat. The same is, of course, true of beef and mutton.

A series of most important experiments, started in Dr Hammond's laboratory in Cambridge and continued in New Zealand, was made to test the effect of feeding on the development of the carcass of pigs and lambs. They showed in the most striking fashion that poor feeding not only stunts, but also delays indefinitely and, if continued long enough, permanently prevents, the full development of the later-developing and best parts of the carcass. If a bacon pig is underfed for half its short life and then given a full ration, this 'low-high' pig will certainly put on weight. But the important point is that the skeleton and muscle will not grow as they would have done if they had had the opportunity at the right time, and the extra food will be used mainly to lay on fat (McMeekan, 1940*c*, Pl. 27).

Growth potential

The moral of this is that growth potential is not a thing that one can speed up and slow down, as one can accelerate or retard a chemical process, and get the same result in the end. If one does not use the full potential all the way along one does not get

full development. One may get the same ultimate weight but one will get a carcass that is different in shape and in composition. Now we have an idea of what is implied by a well-grown pig. If it is also fat, that should be 'finish' added when the correct framework of bone and muscle has grown to the desired size.

It may seem that it is a long step from bacon pigs and prime beef or mutton to man. Not so long a step perhaps.

An old diagram, drawn by Stratz and widely reproduced, e.g. by Brody (1945), of the change in shape that occurs in man during growth shows that, in essentials, the change is the same as in the pig. Development is from the cephalo-thoracic region forwards and backwards and the hind-quarters mature last. If the rate of growth is sufficiently slowed the adult is not only small but underdeveloped, with normal or nearly normal size head, moderately retarded trunk and relatively short legs.

Let me make quite clear at this stage that this does not necessarily mean that all small adults are underdeveloped. There are still all too many whose small total height is attributable to rickets in infancy. Often they are people with a high growth potential whose parents were quite simply ignorant of any special need for material for calcification of bone. But, apart from these, and possibly differences of build due to variations in endocrine balance, it would be expected on general principles that children continuously underfed would grow into underdeveloped adults. If this is so, a difference in body proportions should be demonstrable in association with differences in height wherever persistent differences in nurture are known to exist. And that, I submit, can even now be confirmed by observation in any city in this country, if one looks for it.

Proportionate growth of children

The graph depicting social gradient in height of schoolchildren between 1927 and 1935 which is to be found in *Food, Health and Income* (Orr, 1937) does not represent the extreme difference at that time. Selection of data for poor urban council schools gave a curve that was still lower. I tried to find recorded data to test my hypothesis about difference in shape, but unfortunately leg length is a measure not often recorded. At that time I found only one set of data (Hansen, 1932) from which similar conclusions had been drawn and which quoted, from an earlier observer, that 'Full typical development in man implies, relatively to body length, short torso, long arms and long legs.' It is a set of measurements of Copenhagen schoolchildren, which included not leg length but sitting height, and deduced that taller children had relatively longer legs and were relatively heavier. (The difference would probably have been more obvious if leg length had been measured.) Anyway, when the Carnegie U.K. Dietary and Clinical Survey was planned at the Rowett Research Institute in 1937, cristal height*, as a measure of leg length, was included in the measurements to be made. Statistical analysis of the data, divided into groups according to expenditure on food, has only recently been completed and the gist of the results is as follows. I am indebted to Mr Quenouille of the Statistical Department, University of Aberdeen, for the information.

The three measurements, height, cristal height and weight were analysed to find out

* Height from the floor to the highest point of the iliac crest.

which predicted most reliably the expenditure group to which a child belonged. It was found that cristal height was consistently better than total height for indicating expenditure group and, for age groups under 12, height was better than weight. In other words, the difference in leg length was relatively greater than that in either total height or weight.

That is to say, this idea was statistically confirmed as far as social gradient is concerned; and confirmed at a time when that gradient was already rapidly diminishing. But I shall not transgress into Dr Weir's field and talk of secular changes in growth at this stage. Instead, I am going to suggest that the consciousness of this difference is universal and so fundamental that it colours both our literature and our art. In romantic literature, the hero and heroine are always long limbed. If the heroine is small, and of course some readers like her that way, it is always expressly stated that she is 'perfectly proportioned'. Conversely, if the villain has to be large, he is also coarse, brutish or gorilla-like. The same is true in art. One will admit I suppose that advertisements, because they aim to use the most popular imagery, might be a good mirror of popular taste. High-class fashion journals depict women with an extreme length of limb, and decorative art does the same for both men and women. One may think some of the drawings absurdly elongated. Yet, compared with photographs of ballet dancers and mannequins there is, in fact, no great difference in proportions. A very small increase in the ratio of leg length to total height has a surprising effect on appearance. Conversely, a proposition may be proved also by the Euclidean device of *reductio ad absurdum*. When the artist wishes to depict the lower orders, as such, or the comic, he draws people with exaggeratedly short legs and makes them fat, with results which suggest the 'low-high' pig.

Growth target

All this, to my mind, implies that there is a general awareness of a growth target which is physiologically sound because it means full development. And, quite unconsciously no doubt, because the later-developing parts, the legs, suffer most in underdevelopment, their elongation and perfection of shape become the symbol of perfect growth.

The next question is whether this symbolism is purely aesthetic (with a possible tinge of snobbishness) or whether it has some other connotation. And that brings us to health.

What is meant by fitness

It would of course be absurd to suggest that all tall people are necessarily healthy or that all small people are sickly. It might be, and indeed it is, argued that there is no tougher adult than the undersized survivor from the slums of some of our cities. But that is an argument that cuts both ways. If they have survived the tempest of the slums because they are specially tough, then it seems a pity that so many of the breed die young and that they are not beautiful as well as tough when they do grow up. If I argue on these lines I am given one of two answers. The first is that, of course, they are the product of 'natural selection' and therefore obviously superior, the doctrine of the 'survival of the fittest'. The greatest disservice, perhaps, that scientists ever did for mankind was to produce this association of ideas between natural selection and

improvement. The entirely false analogy it suggests with such metaphors as winnowing chaff from grain; the altogether false idea that 'the survival of the fittest' implies some absolute virtue and not merely fitness to survive in a given environment, whatever it may be, have done much to hamper and nothing to promote either agriculture or human progress. Progress in any branch of science means increasing control over nature; this is true everywhere from the tilling of the soil to the splitting of the atom: to be content to abandon a large section of the population to the mercy of a man-created, evil environment is so unscientific as, fortunately, to have something quite unreal about it.

The second answer I get is that size has no virtue in itself and that what we want is 'strength'. It is difficult to pin this school of thought down to an exact definition of strength. If they mean 'brute' strength in the general sense of ability to toss cabers and lift heavy weights, or similar muscular performances, that, I should think, is of minor, and rapidly diminishing, social significance, and, in any case strength in that sense is so closely dependent on training that it is hardly worth arguing about. I am reminded of an early attempt to assess physical fitness by measuring vital capacity. The list of persons examined, in descending order of merit, began with boy scouts and ended with beggars and gentlemen.

The idea in the minds of some, at least, of these objectors appears to be that the tall child is something of a 'hot-house plant', a false and dangerous analogy. Obviously if one turns hot-house plants straight out into a cold and unsheltered garden, they will suffer more than plants reared in the open garden, if they can be so reared. But that does not mean that they are inferior or less desirable plants. It simply means that the hot-house is a more desirable environment, for a particular purpose, than the garden. We come back, full circle, to the dandelion and the edelweiss. It all depends on whether we prefer them well grown or stunted and picturesque, if they are picturesque when stunted.

Development in relation to health

Before we leave this question of 'strength', it is perhaps worth while to ask whether height, *per se*, is of any disadvantage in muscular work. An athletic friend of mine thinks it is in football and another suggests that a high proportion of well-known lightweight boxers have come from Glasgow. It would be of great interest to have statistics of the heights and weights of distinguished athletes. So far, I have been able to find only one study, a very recent one (Tappen, 1950) of the world's champion weight-lifters and their records. There are three lifts: the two-arm military press, the two-arm snatch, and the two-arm clean and jerk. The order of weight lifted in the first two is on the average about one and a half times body-weight, and in the third about five times body-weight. In all, the performance is closely correlated with body-weight: coefficients 0.85, 0.82 and 0.80. Since weight and height are themselves correlated, efficiency increases also with height. When weight is held constant, the press lift is hampered by height, slightly but significantly; in the snatch, height has a slight but not significant advantage; in the clean and jerk, the most complicated performance, height has a significant favourable effect on performance. Hence, as far as this goes, height is a slight handicap where the

stance is rigid, and the performance relatively slow, but where speed and agility are required in addition to muscle strength, then height is positively correlated with lift.

So much for feats of muscular strength. Where 'strength' involves also endurance there is indirect evidence that height, weight and performance are correlated. For instance, the lower limit for admission of regular recruits to the navy has been consistently higher than that for the army; and that for the air force (flying personnel) was, at least to begin with, still higher. That meant, of course, that these services were recruited from progressively higher strata of society. Further, within these services, rates of sickness and invaliding decreased as height and weight rose. Put the other way round, rejects, including those who passed the height test and were rejected on medical examination, were, on the average, smaller than those accepted; and, in times of depression, when the supply of labour exceeds the demand, the same is true.

Development in relation to resistance to infection

We have then, so far, no evidence against the view that better development implies greater physical fitness. What other criteria, in the present state of knowledge, can be applied? Morbidity data are few for the general population and it is difficult to disentangle the causes of sickness. Since all the social circumstances, housing, sanitation, spacing of population and hence exposure to infection, as well as education and, on the whole, facilities for prompt medical attention, improve with, and at about the same rate as, growth, it is difficult to judge whether inhibition of growth itself has any effect on morbidity. Examples could, I think, be cited from animal experiments but I prefer to draw such deductions as may be from human populations. Evidence is afforded by the wartime history of tuberculosis. In both world wars, where diet deteriorated to the extent of inhibiting the growth of children, the incidence of tuberculosis rose in proportion. Such an increase might be attributed to simultaneous deterioration in housing, hygiene, isolation and hospital treatment, such as did occur. If we take the two wars separately the deterioration of the environment by destruction of houses, overcrowding, blackout, and failure of isolation were incomparably greater in the second than in the first, but the effect on tuberculosis, especially in Germany, was much less. Not only was the actual rise less, but the transition from the benign and chronic to the virulent miliary disease (which destroyed the immunological theory of racial immunity), did not occur in the general population, but was seen only among starving refugees and displaced persons. It looks as if underfeeding, which produces underdeveloped people, also interferes with the processes which determine immunity or susceptibility to tuberculosis.

The study of this subject is greatly hampered by the impossibility of recording accurately both attack rate and mortality rate; and even when we have both, we so often do not have age incidence, and that may have a decisive effect on mortality. For instance, infective hepatitis, during the last war, occurred five times as often in British as in Indian troops, but case mortality in Indians was five times that in British troops. Mortality rates were therefore similar (Witts, 1947 *a, b*) and, if only mortality rate and not also case mortality were known, it might be concluded that there was no difference between the two populations at risk. But the attack rate was probably governed by

previous exposure and acquired immunity; the case mortality by the health, probably the nutritional state, of the men. It appears likely that similar sequences of events account for much excess mortality in poor children early exposed to acute infection, and apparent toughness of the survivors in later life.

Let us look at another indication, namely morbidity rate for bronchitis, concerning the same children in the Carnegie U.K. Dietary Survey on whom the physical measurements were made (Table 1). We find the longer-legged children suffered less bronchitis than the short at all ages. Since there is neither complicating immunity mechanism nor specific cure for bronchitis, we might argue that the constitution built up when the complete harmonious pattern of growth is unfolded is, in some way, superior to that associated with inhibition of growth, however slight.

Table 1. *Percentage incidence of bronchitis*

(Unpublished data of the Carnegie U.K. Clinical and Dietary Survey, 1937-9)

Age (years)	Weekly food expenditure per head of family			
	Up to 5s.	5s. to 7s.	7s. to 9s.	9s. or over
Boys				
0-4	20.5	15.4	10.3	4.3
5-9	10.6	11.4	15.0	6.4
10 and over	5.0	4.0	3.2	2.1
Girls				
0-4	17.1	11.1	7.1	—
5-9	8.4	6.7	3.4	4.3
10 and over	4.1	2.8	1.4	1.5

The trend of evidence then is that the better-fed and therefore better-developed children and adults are 'fitter', measuring fitness by muscular strength, and 'healthier', measuring health by absence of morbidity where we can get a picture in which the complications can be at least partly resolved.

We cannot go much further in this analysis at present. It would be of the greatest interest to be able to trace accurately the further history of well- and ill-grown people in terms of living and dying and causes of death. I can sum up the general picture (Table 2), in terms of social class, which connotes a general difference in standard of perfected growth.

Table 2. *Mean age at death of males who have completed 16 years of life*

(Computed from mortality rates, Registrar-General, 1938)

Social class	...	1	2	3	4	5	All
Mean age at death (years)	...	65.3	65.6	60.4	60.9	60.3	61.4

This shows that, for those who have avoided death in childhood, there is a difference of 5 years in mean age at death between the highest and lowest social classes. An analysis of the reasons, in terms of certified causes of death, is not strictly relevant to this discussion, and I will conclude the argument with one further point. The 'low-high' pig, first stunted in growth and, when it is then well fed, becoming obese rather than 'finished' will be remembered. This, I think, has its parallel among human

populations, where privation increases with size of family and some degree of comfort is attained only after growth has ceased. It is, of course, most obvious where adult occupation is not strenuous. That is one form of obesity. The other is that of the well-fed, well-grown person who is vigorous and athletic in youth and then sits back into the physical inactivity of an office and the comfort of a motor car, but continues to eat about as much as he did when young and active. Table 3 (Keys, 1949) shows the cost of obesity in terms of the weighting of life insurance premiums.

Table 3. *The cost of obesity (America)*

(Life insurance premiums weighting)

Height (in.)	Premium				
	100 Weight (lb.)	115 Weight (lb.)	129 Weight (lb.)	141 Weight (lb.)	154 Weight (lb.)
60	90-169	170-180	181-195	196-209	210-220
64	98-180	181-194	195-206	207-223	224-236
68	110-198	199-214	215-227	228-245	246-259
72	126-219	220-236	237-252	253-268	269-284
76	142-246	247-263	264-283	284-298	299-314

The moral is not quite the same for both groups. The obesity of the well-grown can no doubt be debited to gluttony and sloth, but I doubt whether that of the ill-grown can be prevented except by the continuation of a spartan regime throughout life, which seems a bit hard. The real answer is good feeding in youth, disciplined eating and habits in later life.

SUMMARY

To sum up briefly: we have seen that there is a physiological basis for preferring tall and long-legged people because, in general, that type represents completion of growth and appears to connote a certain superiority of constitution. I have suggested that there is an awareness of merit in this type which is reflected in literature and art. It is reflected also in the utilitarian sphere of selection for employment. This is rational up to a point, but even where robustness and muscular strength are of little or no immediate importance, there is still a tendency to discriminate against the short, and still more against the generally small person unless he is exceptionally gifted. If one is heavy, one may get by on the basis of being a 'solid' man, but that may have its penalties too.

This discrimination may obviously be of considerable social importance and it would be interesting to have an analysis of mental ability, or of socially valuable performance, in relation to attained physical growth.

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Phases of Postnatal Growth

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The development of the human young from birth to maturity can be divided arbitrarily into a series of stages each characterized by peculiarities of physiology, nutritional requirements, physical proportions, and social adaptation. Whilst it may be convenient to refer to these phases in terms of chronological age, there is a considerable physiological variation between individual children and between the two sexes in the age at which each successive stage is reached and passed. These individual differences become more obvious clinically in the later age groups, but substantial developmental differences in osseous development can be demonstrated radiologically even amongst groups of normal infants and pre-school children of the same age. Mean standards of bone age or osseous development have been established by Todd (1937) and Greulich & Pyle (1950) for children of both sexes from birth to the age of 18 years and are valuable in assessing the developmental age of a particular child.

Methods of assessment

It is the purpose of the present communication to outline briefly the phases of postnatal growth which may be recognized, and to show that these do not bear a consistent relationship to chronological age. Whilst it is not intended to discuss in detail the various methods that have been adopted to assess and analyse growth, it will be realized that our present knowledge has been built up in two ways, the 'cross-sectional' study of large numbers in each age group, and the 'longitudinal' or continued study of the same individuals over a period of years. The methods used in assessing the individual child have evolved from the crude comparison of individual height and weight with mean values obtained from the examination of large numbers of healthy children in each age group. A significant advance was made by the construction of weight-height-age tables for pre-school children (Woodbury, 1921) and schoolchildren (Baldwin & Wood, 1923) in which the necessity for correlating height with weight was clearly recognized. Subsequently height and weight indices (Sutcliffe & Canham, 1950) and percentile ratings have been employed with the purpose of indicating the child's relationship to the mean and to the normal range in each age group, and it has been found that the child tends to retain his position within the group in relation to any particular measurement during childhood (Meredith, 1937a, b). At the present time, the most satisfactory method of recording individual growth in childhood is probably the Wetzell grid (Wetzell, 1943). From this graphic record in which height and weight

are correlated it is possible to see whether the child's progress falls within the expected channel for age and sex, and deviations from the height-weight relationship characteristic of children of particular body-build become obvious. The methods of assessing growth and progress in infancy are rather less satisfactory than for schoolchildren, partly owing to the greater difficulties of accurate measurement. The fallacies of adopting weight as the sole criterion, and of comparing the individual infant's weight curve with a composite weight curve are so obvious that they should not require emphasis if they were not so frequently overlooked and so often responsible for unnecessary anxiety. Thus the birth weights of healthy full-term infants may vary by more than 100 %, and this variation in birth weight is likely to be reflected to some extent in the weight curve subsequently. Indeed, Illingworth, Harvey & Gin (1949) have shown that in each age group from 6.5 to 13 years, children of higher birth weight are on the average significantly heavier and taller than those of lower birth weight.

Phases of postnatal growth and development

The *neonatal period*, arbitrarily taken as the 1st month of life, during which the infant is adapting to extra-uterine existence. During the first 4 or more days the infant commonly loses weight, the loss depending to some extent on the birth weight, establishment of lactation, capacity for vigorous sucking, and other factors. The birth weight is usually regained by the 10th day.

Early infancy, or the *suckling period* preceding the eruption of the first teeth, the lower central incisors usually appearing at approximately 6 months of age. This period is marked by rapid growth, the birth weight often being doubled, or more than doubled, by the age of 6 months. This is reflected in the high caloric requirement of 50 Cal./lb., compared with the estimated figure of 20 Cal./lb. for an adult working male.

Later infancy, from 6 months to 2 years of age, during which the eruption of the first dentition is completed, the diet is gradually enlarged by the addition of solids from one almost exclusively of milk, and the acquisition of speech and upright posture involves a corresponding enlargement of physical and intellectual horizon.

The fourth or '*pre-school*' period is one demarcating by social custom in this country the ages 2-5 years, though developmentally it would be better if extended to 6 or 7 years, to correspond both with the eruption of the second dentition and to changes in physique which are observable clinically. Whilst the home remains the central nidus of culture and infection, the pre-school child is extending his social contacts with the consequent likelihood of contracting the common infections of early childhood from outside sources. New skills and more systematized play techniques are rapidly acquired, with corresponding effects on intellectual and muscular development.

Childhood proper, lasting from the age of 6 or 7 years to the onset of puberty. During this period the major part of the second dentition is established. In the emotional development of the child this period is sometimes described as 'latent', whilst physical growth in general tends to be relatively slow and uniform until the onset of puberty is approached. The period is, however, associated with an acceleration in growth of lymphoid tissue, and failure to recognize the physiological nature of tonsillar enlargement in childhood may lead to unnecessary tonsillectomy.

Pubescence, which may be defined as the period beginning with the earliest evidence of either genital development or appearance of secondary sexual characters such as pigmented pubic hair, or breast development in girls, and ending with the menarche (first menstruation) in girls, and with advanced and uniform genital development in boys. It has been estimated that pubescence lasts approximately 1·2 years in boys reaching pubescence at 13·5 years, and approximately 2·5 years when pubescence begins at 10·5 or at 14·5 years (Ellis, 1948*a*); for girls, the figures given by Hogben, Waterhouse & Hogben (1948) indicate an average duration of pubescence (onset of breast development to menarche) of 2·6 years. Girls were also found to show onset of pubescence 2–2·5 years earlier than boys (Cawley, Waterhouse & Hogben, 1949).

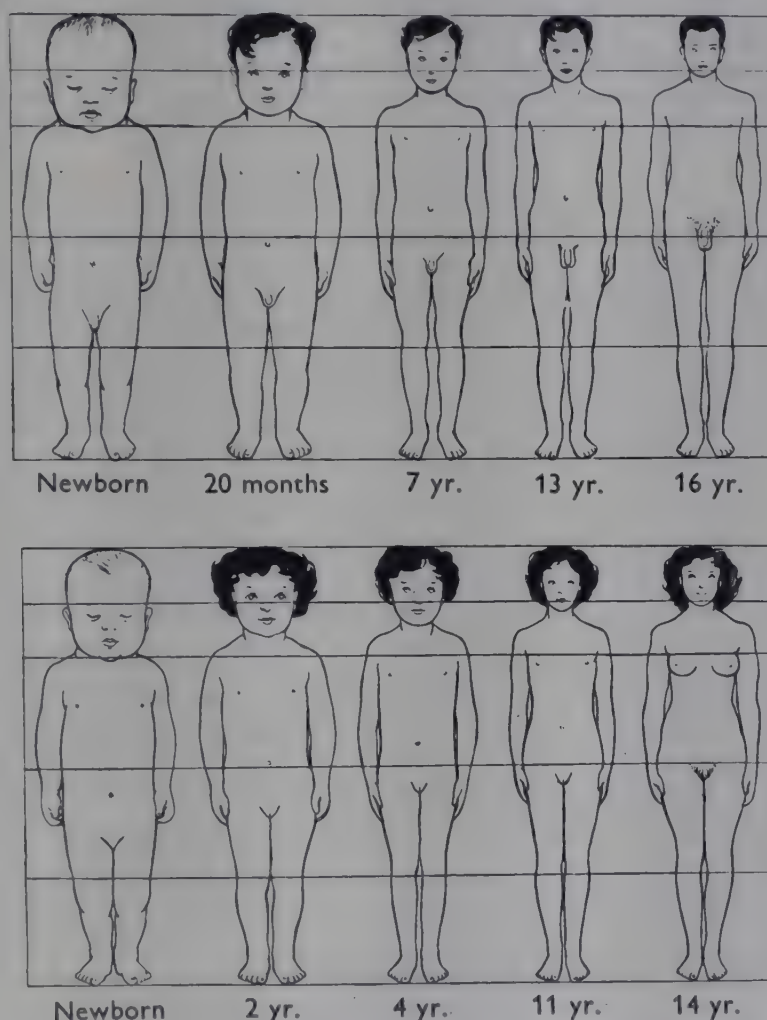


Fig. 1. Proportions from birth to adolescence (Ellis 1947).

Adolescence is the period immediately following pubescence and culminating in fusion of the epiphyses of the long bones with consequent cessation of growth. In a large-scale study of children and adolescents, Simmons (1944) found that terminal height might be reached as early as 15 or as late as 20 years in boys, and in girls as early as 14 or as late as 18 years.

Physical proportions

The *relative physical proportions* of each of these earlier phases of development are best illustrated diagrammatically (Fig. 1). It will be seen that in the newborn infant the head represents approximately one-quarter of the total length and the legs approxi-

mately three-eighths. The abdomen and trunk are relatively large and the limbs short. In adolescence, in contrast, the head represents only one-seventh of the total length and the legs one-half. The proportions have changed from the brevilinear ones of infancy to the longilinear ones characteristic of early adolescence, whilst the umbilicus has risen from well below the midline to well above it.

In addition to the changes in skeletal growth revealed by external measurement or radiography, there are characteristic differences in the rate of growth of different tissues (Scammon, 1930). The growth of nervous tissue is most rapid during the first 2 years and minimal after the age of 8 years; the growth of lymphoid tissue is maximal during middle and later childhood; whilst the growth of the gonads after the 2nd year is minimal until the onset of pubescence, when it shows rapid acceleration. The rate of mental growth has not been found to show any close correlation with the abrupt changes in rate of skeletal growth associated with increasing maturity (Abernethy, 1936).

Sexual maturation

The period of sexual maturation requires special consideration by those concerned with the nutrition and health of the schoolchild, since rapid acceleration in growth is likely to be reflected in changes in appetite and nutritional requirement. A number of the more detailed growth studies have been carried out on girls and related to the onset of menstruation (e.g. Shuttleworth, 1937, 1938) since, although the first menstruation does not necessarily correspond with onset of ovulation and is commonly followed by a period of relative sterility, it provides an easily defined point in time which may be taken to indicate the beginning of adolescence. It has been found that whilst there are certain differences in growth pattern between groups of girls reaching the menarche early or late, there is in general a maximum increment in standing height during the 1 or 2 years preceding the menarche, followed by rapid deceleration in height increment until terminal height is reached. This is well illustrated by Shuttleworth's (1937) method of superimposing the growth curves of groups of girls menstruating at different ages or of groups in which the maximum annual growth increment occurred at different ages. By plotting the growth-curves of the various groups so that the age of menarche falls in the same vertical line, it becomes obvious that the growth pattern relates more closely to puberty than it does to chronological age.

Since the pre-adolescent spurt of growth is related to the menarche, it is necessary to emphasize the wide variation in age at which the first menstruation may occur. Thus in a series of 470 healthy English women age of menarche was found to vary from 9.5 to 18 years, and in a smaller Nigerian series from 11.5 to 17.5 years. In both instances the distribution curves showing the percentage reaching the menarche in each year of age (Fig. 2) were of approximately similar shape, though the mean of age of menarche in the English series was 13.7 years and that in the Nigerian series 14.2 years, giving no support to the popular view that puberty occurs earlier in the tropics than in temperate zones. Other English figures comparable to those quoted are given by Wilson & Sutherland (1949) and Hogben *et al.* (1948). With boys, it is possible to recognize with reasonable accuracy three successive stages of maturity, which I have described as non-pubescent, pubescent, and adolescent, on clinical criteria which have

been detailed elsewhere (Ellis, 1946). Owing to the difficulty in classifying certain borderline cases, these three gradings have been used in preference to the five described by Greulich, Dorfman, Catchpole, Solomon & Culotta (1942), whilst the criterion of

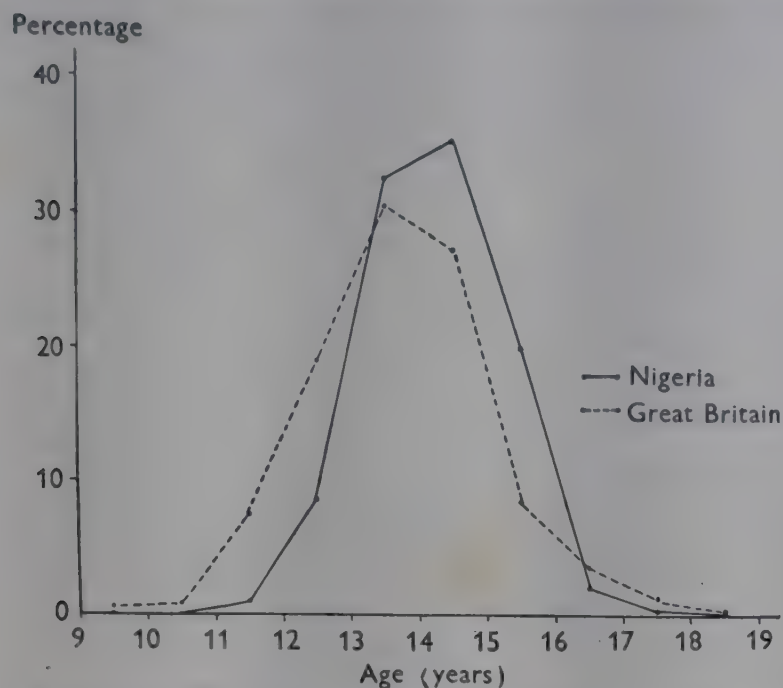


Fig. 2. Percentage of girls reaching the menarche in each year of age, Nigeria and Great Britain (Ellis, 1950).

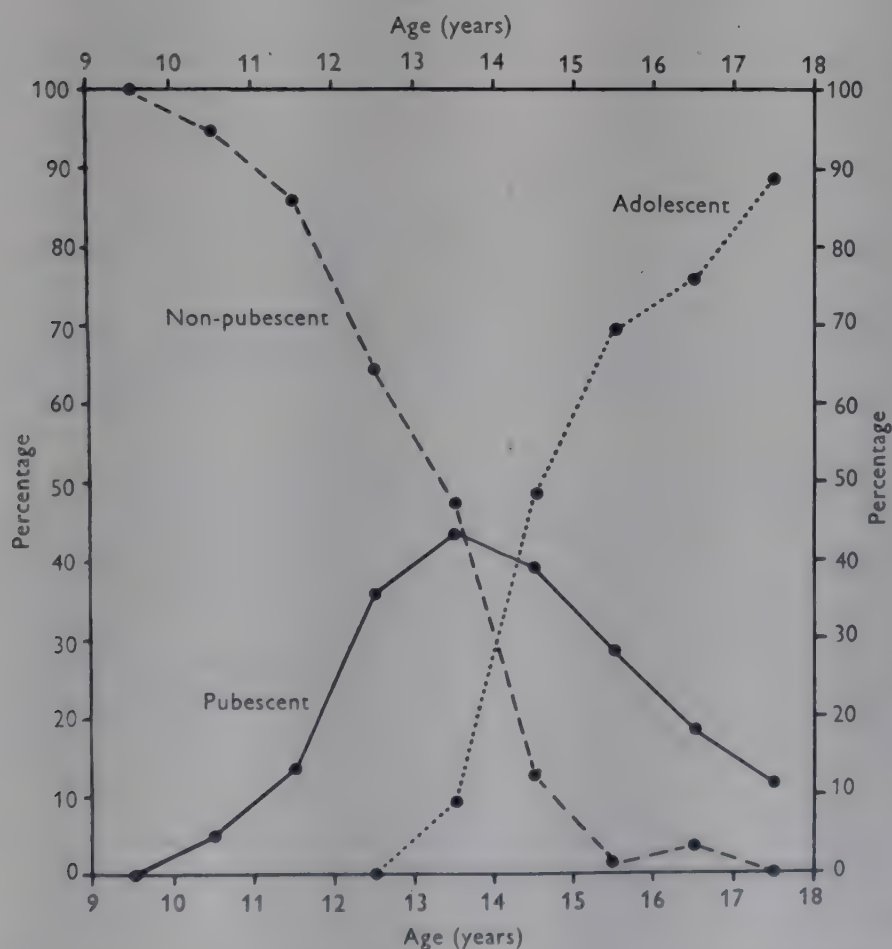
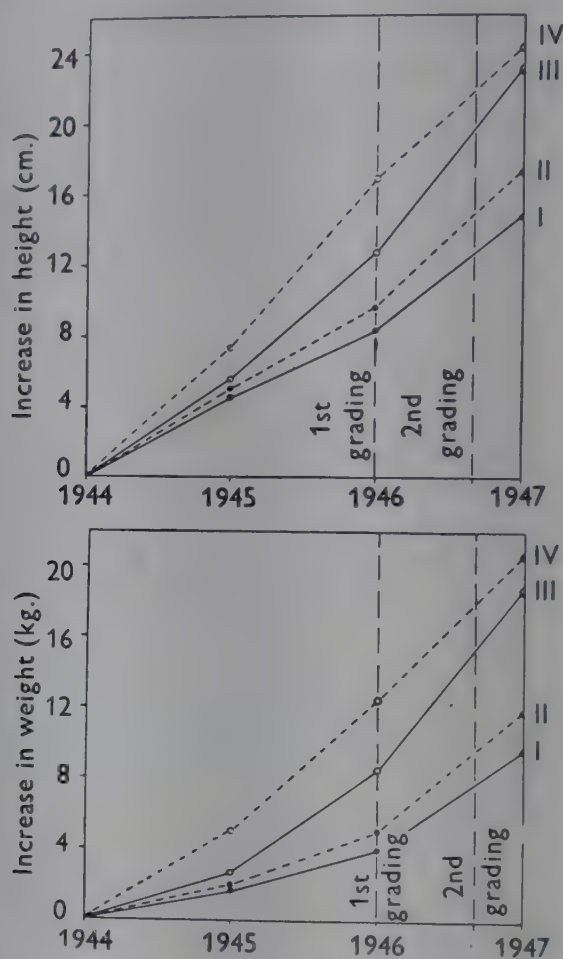


Fig. 3. Percentage of boys graded as non-pubescent, pubescent and adolescent in each year of age (Ellis, 1946).

adolescence used by Richey (1937), namely appearance of axillary hair, has been felt to be inadequate. By applying this maturity grading to boys between 9 and 18 years of age, the percentage non-pubescent, pubescent and adolescent in each year of age is shown in Fig. 3 (Ellis, 1948*a*). The number graded as non-pubescent was found to fall from 100 % at 9.5 years to 50 % at 13.1 years and 0 at 17.5 years; the figures also illustrate the effect of raising the school-leaving age from 14 to 15 years. Thus at 14 years



Figs. 4 and 5. Annual height and weight increments of boys in four maturity gradings: I, non-pubescent on first and pubescent on second grading; II, pubescent on first and second grading; III, pubescent on first and adolescent on second grading; IV, adolescent on first and second grading (Ellis, 1948*a*).

there are as many non-pubescent boys as adolescent, and approximately 40 % of boys are pubescent, whereas at 15 years approximately 60 % of boys have attained adolescence and less than 10 % are non-pubescent. Since each successive phase of maturity is associated not only with differences of growth rhythm, but also of physical performance (Espenschade, 1940; Ellis, 1948*b*), this has a practical bearing on the composition of groups of boys reaching the labour market, and also on the school-meals service which is now providing for a substantially larger number of boys in whom rate of growth is at a maximum.

In order to assess the rate of growth associated with each successive stage of maturity, boys living in a residential school were graded at the beginning and end of a 9-month period and divided into four groups: (1) those non-pubescent on first and pubescent on second examination; (2) those pubescent on first and second examination; (3) those pubescent on first and adolescent on second examination and (4) those adolescent on

first and second examination. The mean annual increments in height and weight of each group were assessed for the 2 years preceding and the year following the first grading. These figures (Figs. 4 and 5) and other data included in the same study (Ellis, 1948*a*) demonstrate an acceleration of both height and weight increment preceding the clinical manifestations of pubescence and continuing through pubescence, a more rapid gain in both height and weight associated with the passage from pubescence to adolescence and continuing into early adolescence, and subsequent deceleration in height increment noted in older adolescents.

In conclusion, I would like to emphasize again the importance of maturity grading during the growth period, rather than the too rigid concern with chronological age which is evident both in the present Education Act and in the legislation for protection of juveniles in employment. Widdowson (1947) has shown the enormous variation in food consumption of children within the same year-age groups. It would be of great interest to determine whether maturity grading applied to the subjects of such a study would show more consistency in caloric intake in relation to the stage of maturity reached and rate of growth than was revealed by age grouping alone.

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Human Foetal Growth

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The general picture

After fertilization, the dividing ovum floats in the intra-tubal and intra-uterine fluids for a few days and then becomes embedded in the uterine wall. Supplies of water, oxygen and nutrients for the embryo are assured by the trophoblast in contact with maternal blood and soon by the placenta. Within about 3 weeks of implantation the beginnings of the foetal circulation are established. From this point, until a late stage of pregnancy, the supply system seems to keep well ahead of demands. At first, embryonic differentiation is more important than growth in size, and all the main organs and tissues are formed before the end of the 1st trimester, when the foetus weighs only about 20 g. I can only touch on an interesting aspect of this early stage, the role of organizer substances and their relation to nutrition. Chemical substances control the differentiation of various parts of the embryo. Interference with organizer effects during critical phases of development, e.g. by lethal genes, the action of some viruses, or even by local injury, is probably the cause of some of the so-called congenital abnormalities. Certain vitamins seem to behave as organizers during specific phases of development. Warkany (1945) has produced a number of developmental defects in foetal rats by restricting riboflavin intake to a critical degree during a short critical period of gestation, and similar effects have been noted in pigs as a result of vitamin A restriction (Hale, 1935). A detailed account of organizer action is given by Needham (1942); a lucid summary can be found in Corner's (1944) remarkable 'embryologist's essay on man'.

The initial phase of differentiation is succeeded by a phase of maturation and growth in size. Flexner, Cowie, Hellman, Wilde & Vosburgh (1948) showed that placental permeability to radioactive sodium increases some seventyfold between the 9th and 36th weeks of pregnancy, followed by a decline, towards term, associated with degenerative changes in the placenta. Although it seems that the supply mechanism becomes markedly less efficient near term, at the same time the greatest actual bulk of new foetal tissue is being produced. During the last few weeks of pregnancy, therefore, the rate of growth of the foetus is more likely to be affected by limitation of supplies.

It should be noted, however, that the nutritional demands of the foetus are never very large. At mid-pregnancy the heat of combustion of the foetal body is only about 160 Cal. and at term it is only about 4000 Cal. (Fig. 1, curve C). The protein content of the full-term foetus is about 360 g. and the calcium content about 25 g. Even if the efficiency of conversion of nutrients into foetal tissue is low, the absolute demands by the foetus itself on the maternal supplies cannot amount to much. Too much may be made of the concept of foetal competition for supplies, which cannot be serious unless the mother's reserves are low and she is eating a diet barely adequate for herself. (The direct demands of the foetus are not, of course, the whole story of maternal requirements during pregnancy.)

Normal foetal growth

Fig. 1, curves *A* and *B*₁, shows average growth curves for length and weight of the type quoted in most standard texts. Both are fairly smooth curves. The inflexion at mid-pregnancy in the crown-rump length curve is probably due to a slight slowing of trunk growth as the growth of the limbs accelerates. The weight curve is shown as

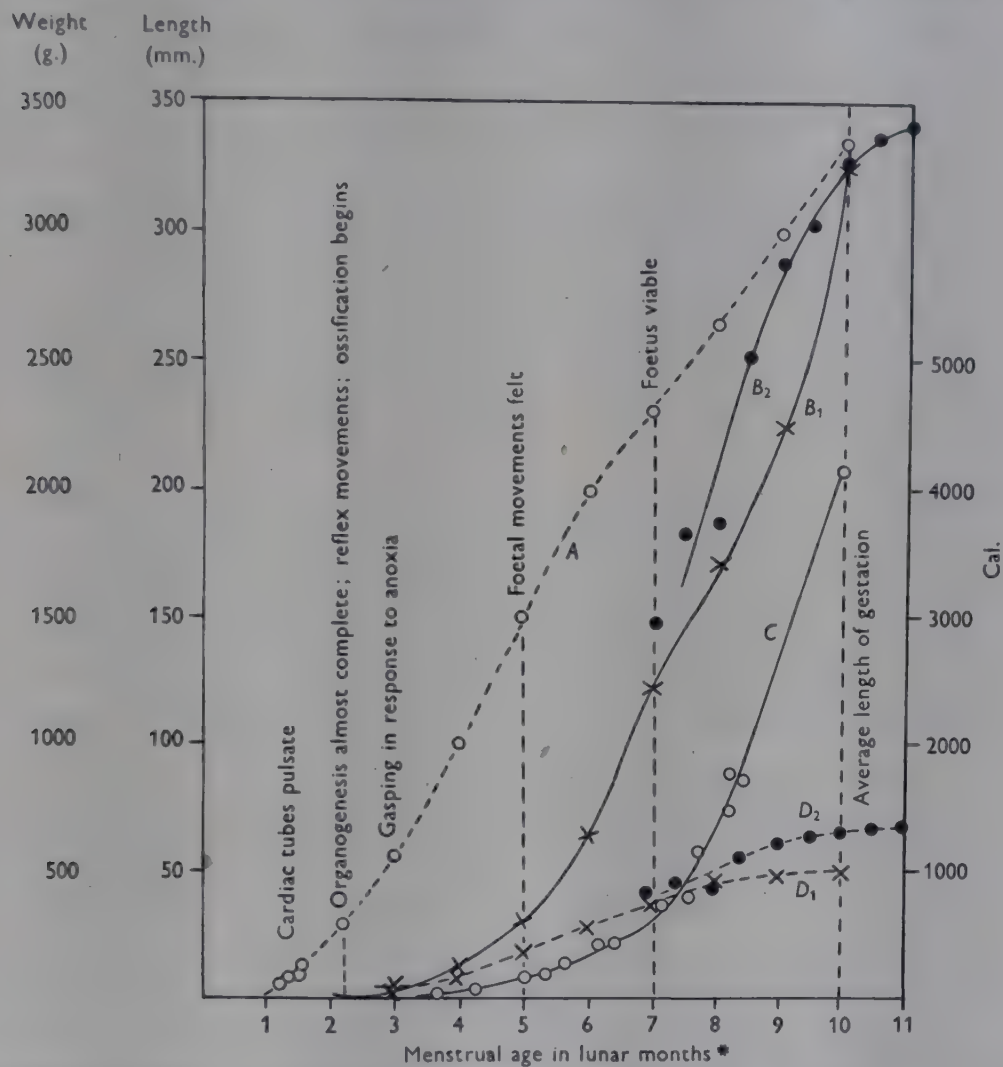


Fig. 1. Growth and development of foetus and placenta. *A*, $\bigcirc$ - - - $\bigcirc$, foetal crown-rump length, mm. (Leitch, 1950); *B*₁, $\times$ — $\times$, foetal weight, g. (Leitch, 1950). *C*, $\bigcirc$ — $\bigcirc$, foetal heat of combustion, Cal. (Leitch, 1950). *D*₁, $\times$ - - - $\times$, placental weight, g. (Hecker, 1866). *B*₂, $\bullet$ — $\bullet$, foetal weight, g.; *D*₂, $\bullet$ — $\bullet$, placental weight, g., Aberdeen Maternity Hospital married primiparas, 1948–9 (excluding twins and gross deformities).

* Menstrual age connotes the interval between the 1st day of the last menstrual period and the day of delivery.

steepest during the final month of pregnancy, and if this is correct the foetus puts on about 0.6 kg. (1.3 lb.) during the last 2 weeks, which is not in accordance with obstetric experience. I have inserted data from Aberdeen Maternity Hospital for first single births to married women (curve *B*₂). This confirms the finding of Hosemann (1948), that the curve for average birth weights is sigmoid. Hosemann's data suggest that average birth weights actually decline after the 11th lunar month. Though such curves, constructed for average birth weights, do not necessarily prove that the growth of the individual foetus follows the same pattern, there is reason to believe that the rate of growth becomes less during the final stage of gestation. Clinically, there is no evidence

of exceedingly rapid foetal growth during the terminal phase. Curves D_1 and D_2 show that the growth of the placenta slows down towards term, and the data of Flexner *et al.* (1948), already cited, suggest that its functional efficiency also declines during the final stages. If the foetal supply mechanism is becoming less efficient, it would be surprising if growth continued unimpaired. Incidentally, curve D_1 is constructed from the only figures I have been able to find for placental growth, and these are 84 years old. Curve D_2 shows average placental weights in the group of Aberdeen cases.

Barcroft (1946) has said that such total-growth curves give only limited information, since they conceal the individual characteristics of the growth patterns of organs and tissues. Knowledge of organ and tissue growth in the human foetus is limited. Most of the essential organs, and particularly the brain, form a large proportion of the total body size at first, the proportion declining as gestation proceeds. Changes of body constitution in response to nutritional influences, such as those reported for the foetal lamb by Wallace (1946) have not been worked out. Clatworthy & Anderson (1944) have summarized knowledge of the differential growth rates of organs and tissues in the human foetus.

What is the scatter round the average curves shown in Fig. 1? Barcroft (1946) has given data indicating that the dispersion of weights of foetal lambs is relatively small during the greater part of gestation, but during the last 2 or 3 weeks there is a remarkable increase of scatter, so that the newborn lamb can weigh anything from about 2 to 7 kg. In his own words: 'These facts are, I think, best explained by supposing that as term approaches some of the foetuses have faltered.' Wallace (1948) and Thomson & Thomson (1948-9) have shown that sharply contrasted maternal diets during the latter part of pregnancy produce a marked difference in the average birth weights of lambs. The latter authors, however, point out that there is a considerable overlap of the individual birth-weight data for the two nutritional groups (for singles and twins separately, all sheep being of the same breed), so that some poorly fed ewes produce lambs as big as some well-fed ewes. Data for the variations in human foetal weights throughout gestation do not appear to have been published. Hosemann (1948) gives curves showing the distribution round the median during the last trimester; it would appear from these that the dispersion is no less at 28 weeks than at 40 weeks. My obstetrical colleagues tell me that they have not been impressed by any similarity of size between very immature foetuses of allegedly similar gestational age. There is therefore a possibility that, unlike the sheep foetus, the human foetus shows marked differences of growth throughout gestation.

The variation in birth weights of full-term infants is certainly striking. Fig. 2 gives the frequency distribution of full-term birth weights among 766 Aberdeen primiparas whose lengths of gestation were considered to be reliably known.

Technical difficulties in assessing growth rates

The literature is full of information on birth weights, but the data are almost never adequately broken down by length of gestation, so that it is very difficult to draw conclusions as to foetal growth rates. Fig. 1 indicates that quite small differences of foetal age can introduce large differences in size. Knowledge that the mean lengths of

gestation in two groups to be compared are similar does not help much, since growth near term is not steady in relation to time.

The determination of the length of gestation in human patients is difficult. The exact date of fertile intercourse is seldom known, and the assumption that fertilization takes place 14 days after the 1st day of the last menstrual period is only roughly true, even in the absence of menstrual irregularities. Apart from such inherent difficulties, ordinary practice is often very inexact. The expected date of delivery is worked out by rule of

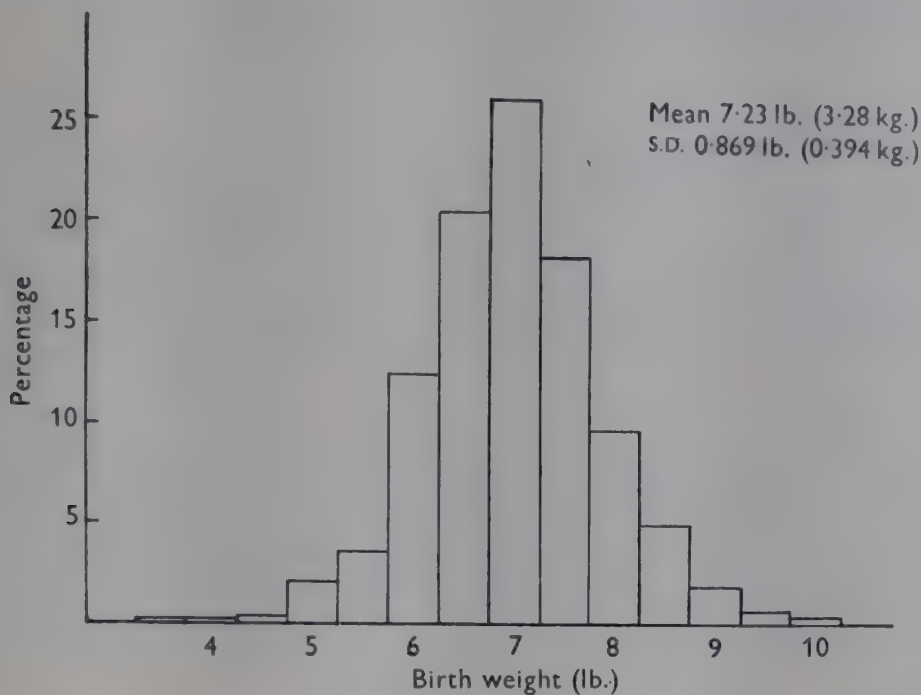


Fig. 2. Distribution of birth weights among babies of 766 Aberdeen Maternity Hospital primiparas delivered at 280 ± 7 days. Twins, seriously deformed infants, and cases where the menstrual age (see Fig. 1) was doubtful have been excluded.

thumb from the patient's stated date of last menstruation. Some patients do not know that the 1st day is wanted; many do not remember exactly; and some give the wrong date in an effort to conceal pre-marital conception. When the child is born, its maturity is worked out by rough and ready mental arithmetic relating the date of delivery to the expected date; and if the birth weight is unusually high or low an arbitrary correction is quite likely to be made, on the assumption that 'the dates must be wrong'. The procedure we have adopted in our cases is to cross-question the patient on the reliability of her menstrual date, to check her statement, when possible, against the clinical findings during pregnancy, and to use exact methods for calculating the interval between the last menstrual period and the date of delivery. 'Uncertain' cases are not included in the series reported here. Though the exactness of our methods is recognized to be relative only, it is difficult to see how greater accuracy can be achieved in routine practice, and it is probable that the interval is correct to within a few days in most of the cases.

Finally, it should be noted that weight is highly labile, and is, in some ways, the least satisfactory measure of growth. We badly need reliable data on the skeletal measurements of babies of known gestational age.

Factors causing variations of foetal growth

Part of the great variation in birth weight observed in the full-term foetus is likely to be due to errors in the estimation of foetal age, despite the precautions noted above. But the standard deviation of the observations is about 1 lb. (see Fig. 2), and it is most unlikely that errors in the estimation of foetal age could account for more than a small proportion of the variance. All the Aberdeen infants were weighed on lever balances in hospital. It can be concluded, therefore, that most of the apparent variability of growth really exists. The possible causes will be examined.

Genetic factors

Sex is the only inherited factor about which we know much. Boys grow slightly faster than girls. In the Aberdeen series the mean weights at 280 ± 7 days were: boys, 7.38 ± 0.882 lb. (3.35 ± 0.400 kg.); girls, 7.07 ± 0.828 lb. (3.21 ± 0.376 kg.). It is likely that a genetically determined growth potential accounts for some part of the variability, but its relative importance is unknown at present.

Maternal age and parity

All the Aberdeen data quoted are for primiparas. Their analysis supports the usual view that any effect which maternal age may have is so slight as to be swamped by other factors. I have not worked out any figures for different parities. The literature suggests that second babies tend to be slightly heavier than first (Martin, 1930-1).

Maternal size

Walton & Hammond's (1938) well-known experiment with Shetland-Shire horse crosses showed that the size of the newborn foals, under conditions of extreme genetic variability, is determined by the size of the mother and her capacity to nourish and sustain the foetus. Baird (1945) showed that human birth weights, for all menstrual ages*, vary according to the height of the mother. I have worked out similar data for full-term infants only. Fig 3 shows that average birth weights (and hence the foetal growth rates) increase as maternal height increases. In an earlier and smaller series, Mr M. H. Quenouille determined for me the regression of birth weight at term on maternal height and width of the pelvis at the iliac crests. He found a small but highly significant correlation with height; the pelvic measurement, however, contributed nothing to the prediction of birth weight. It should be noted that the standard deviations in each height group (Fig. 3) and for all heights together (Fig. 2) are similar. Thus height by itself does not account for much of the variability in birth weights.

Maternal health

Height signifies more than mere size. We have recently been grading mothers appearing at the antenatal clinic in terms of physique and general physical well-being. The assessment, being subjective, is necessarily rough, but the numbers along the

* Menstrual age connotes the interval between the 1st day of the last menstrual period and the day of delivery.

bottom of Fig. 4 show that 70 out of 162 (43·2 %) of tall women were assessed as 'good'; whereas only 14 out of 122 (11·5 %) of short women were in the 'good' category. The most probable interpretation is that well-nourished women are well-grown as well as healthy, whereas the ill-nourished are not only stunted but do not have the appearance of vigorous health. The same figure shows graphically the average birth weights in each physical grade arranged by height. Women graded as 'good' tend to have larger babies,

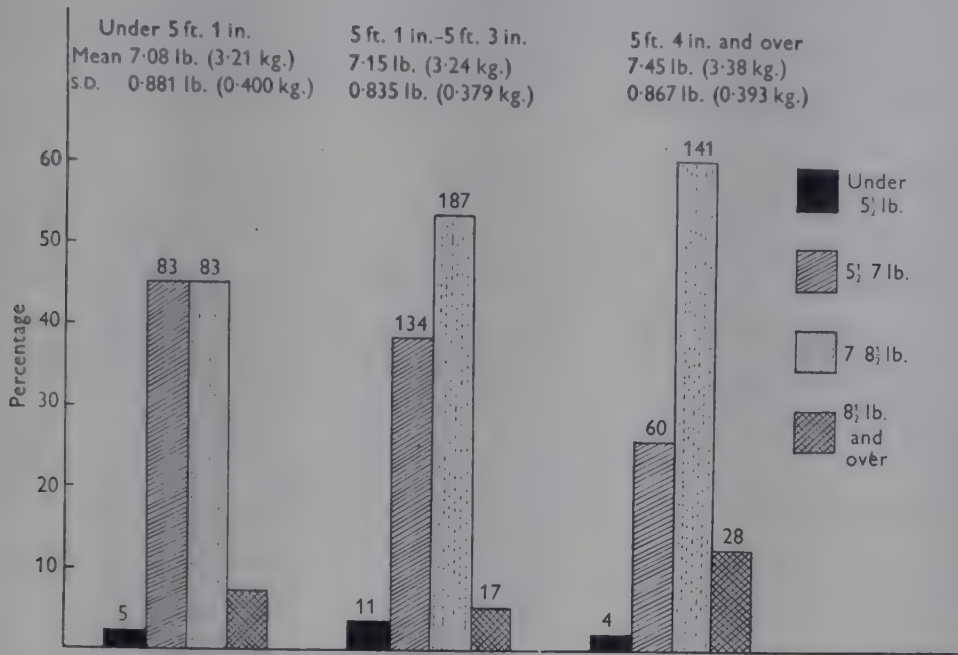


Fig. 3. Maternal height and birth weight at 280±7 days. (Aberdeen Maternity Hospital primiparas.)
Figures above columns are numbers of cases.

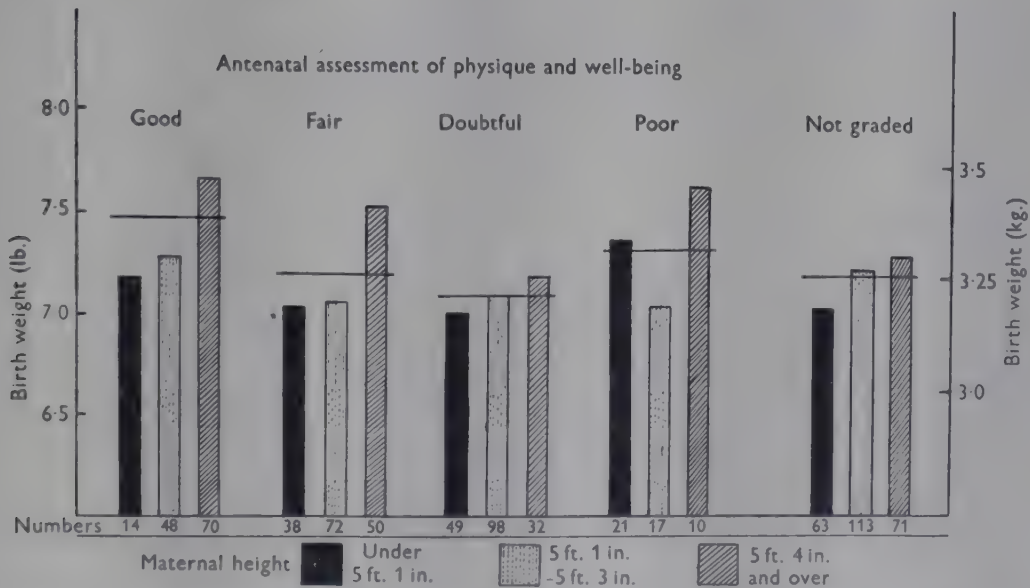


Fig. 4. Average birth weights according to maternal height and physical well-being. (Aberdeen Maternity Hospital primiparas delivered at 280±7 days.) Cross-line in each group represents the mean of the group.

and this is true for each height group separately. Down to the grading 'doubtful' there is the appearance of a fairly regular decrease of average birth weight as physical condition deteriorates. The increase of average birth weights among 'poor' patients is presumably a random variation. The numbers are small.

Pathological conditions

That large babies of low vitality are borne by diabetic mothers is well known (Lawrence & Oakley, 1942). I have not found any data showing whether foetal growth is altered in other pathological states of the mother. Many obstetricians believe that the baby tends to be small when the mother is hypertensive. I have no data on this point, but examination of the hospital records makes it clear that if this is a rule, there are many exceptional cases. I am inclined to think that the weight change of the mother is related to the foetal growth rate and there is support for this view in the literature (Beilly & Kurland, 1945); but once again, if this is a rule, exceptions are frequent.

Maternal nutrition

Stature and physical health are, to some extent, indices of the state of nutrition, past and present, since well-nourished people tend to be tall and in good health. The data presented above suggest that though the maternal plane of nutrition affects the foetal growth rate, it does not account for anything like the total variation. On the other hand, Burke, Harding & Stuart (1943), working in Harvard, claim that birth weights (the gestational ages are not stated) are closely related to the protein content of the maternal diet during pregnancy. Sontag & Wines (1947), also in America, found only an insignificant correlation, and raise doubts as to the reliability of the technique of Burke *et al.* We have been carrying out diet surveys on Aberdeen primiparas by weighing the food consumed for 1 week during the 7th month of pregnancy. Only those mothers considered to have kept reliable records are included in Table 1, the first part of which

Table 1. *Average birth weights of infants arranged according to mother's daily intake of protein (a) and calcium (b) estimated during the 7th month of pregnancy*

(Aberdeen married primiparas, 1948-50)

Daily intake of protein or calcium (g.)	All cases		Delivery at 280 ± 7 days	
	No.	Average birth weight (kg.)	No. of cases	Average birth weight (kg.)
(a) Protein				
Under 70	39	3.28	23	3.32
70-88.9	67	3.22	37	3.23
90 and over	39	3.21	17	3.29
(b) Calcium				
Under 0.800	28	3.33	16	3.25
0.8-1.199	70	3.21	36	3.32
1.200 and over	47	3.22	25	3.21

shows the average birth weights of babies born to women in three different protein-intake categories. No correlation is evident; and the same holds when the diets are grouped according to calcium content.

Diets in Aberdeen are, on the whole, pretty adequate. It may be that the absence of correlation is because almost all our mothers are at least adequately fed at the present time. Under conditions of severe dietary restriction foetal growth may be depressed.

But an extensive literature on birth weights during the war and famine conditions scarcely supports the view that foetal growth is sensitive to anything except very drastic restrictions of diet during pregnancy, and even then the depression of growth would not account for the large variation observable in a well-fed community.

General conclusions

The data presented indicate that there are gross variations in foetal growth under ordinary conditions, of such a degree that one full-term baby may be twice as heavy as another. A small part of this variability is probably due to errors in the estimation of foetal age, but even so a residual real variability of large dimensions remains. Its causes are probably complex. Maternal stature and physical health, both of which are doubtless conditioned to an important degree by nutrition during childhood and adult life, seem to produce a shift in the mean foetal growth rates which is small in relation to the range, and to have little effect on variability. Diet in pregnancy, at all events within the limits of ordinary dietary habits among a reasonably well-nourished population, seems to have an even smaller influence. Claims that foetal growth is readily influenced by changes in diet during pregnancy do not seem to be justified.

These conclusions must be taken in their proper perspective. One may recast them as follows:

(1) The maintenance of a high nutritional level among women generally is probably of considerable importance. If the mean birth weight of the group of babies shown in Fig. 2 were raised by 0.5 lb. (0.23 kg.) without changing the distribution round the mean, an effect which might be produced by prolonged good feeding, the incidence of 'premature' babies, that is, those weighing under 5.5 lb. (2.5 kg.) at birth would be reduced from 2.61 to 0.52 %. (These figures apply only to full-term babies. The overall incidence of prematurity among Aberdeen primiparas is about 10 %. Nutritional improvement would probably not only increase the average rate of foetal growth but also prolong the average gestation period.)

(2) The nutritional level of the individual patient is a poor guide to the probable birth weight of her baby. A change in her diet during pregnancy, within reasonably safe and practical limits, is unlikely to influence the weight of her baby at term by more than a few oz., an effect which has little clinical importance in labour. Good and abundant feeding in pregnancy cannot guarantee the delivery of a big baby, nor restriction of diet necessarily produce a small one. If a small baby is desired in the interests of an easy confinement, it is more certain, and probably safer, to induce labour early than to restrict the mother's diet.

(3) Education of patients in the desirability of good feeding during pregnancy is therefore scarcely worth considering as a means of manipulating the birth weights of individual babies. But such education may have a valuable social effect if carried out routinely on all patients, e.g. through a reduction in the incidence of premature babies.

I have said nothing about the vitality of infants and its relationship to foetal growth. Clinical experience indicates that variations in 'quality' among babies are just as marked as variations in size, and probably of more practical importance. Vitality is difficult to measure, but its study in relation to growth and morphology would certainly be valuable.

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Secular Changes in Growth

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This contribution is printed by title only since it has been submitted for publication as an original paper.

A Comparison of the Vitamin C Content of Vegetable Stew when Prepared on a Large Scale in Open and Pressure Cookers

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In the large compound kitchens of the gold mines of the Witwatersrand, where food is prepared for over a quarter of a million Bantu workers, there is a present trend to replace open cooking by pressure cooking. Of the food consumed, the vegetable stew is the chief, and sometimes the sole, source of vitamin C; however, since scurvy occurs infrequently, the amount of the vitamin provided up to now must have been adequate for protection. All the same, it is desirable that any change in the cooking procedure shall not lower the vitamin C content of the stew. A few investigations of the comparison between retention of the vitamin by both methods of cooking have already been reported in the literature, but they were carried out with relatively small quantities of vegetables. In a stew, in which both vegetable and cooking water are consumed, only the total vitamin content is of importance. In this connexion Noble & Hanig (1948) showed that, during the cooking of the six vegetables examined, the total amount retained averaged about 80 %, though it depended more on the kind of vegetable than on the method of cooking. Chappell & Hamilton (1949), who used a popular type of household pressure cooker, found that for the ten vegetables studied the total retention averaged just under 80 %; the average for the open cooker was about 2 % higher than for the pressure cooker. In the compound kitchens the scale of cooking is very large, and the nutritional implications of the changeover to pressure cooking may be far reaching; the Chief Medical Officer of the Union Corporation group of mines therefore decided that a direct comparison should be made between the retention occurring (a) when the stew is cooked in the usual manner in the open container, and (b) when it is cooked under pressure in a container identical with those it is proposed to introduce in the future.

EXPERIMENTAL

General. In the particular compound kitchen where our investigation was carried out, food is prepared for about 3000 Bantu. The vessels used in each method of cooking are made of steel; they are semi-cylindrical and hold about 170 gal. The usual charge is about 600 lb. vegetables, 160 lb. meat and 70 gal. water. The meat is cooked just before the mixed, minced vegetables are added, heat being supplied from steam led through openings in the base.

Preparation of vegetables. Thoroughly fresh vegetables are delivered to the kitchen

every other day and are stored in a cool dark room. The composition of the vegetable mixture is subject to seasonal variations, but the amounts of green leaf vegetables and of potatoes are kept fairly constant. During the present investigation the mixture used for each cooker was: sweet potatoes 343 lb., cabbage 130 lb., carrots 88 lb. and leeks 14 lb.

After being washed for about 10 min. in a rotating cylinder, two lots of such vegetables were roughly mixed on a cement floor, using a spade, and fed into a mincing machine, where they were quickly reduced to portions about 0.5–1.0 cm. in diameter. (Were the portions larger, certain tribes of Bantu who do not care for vegetables would try to pick out and discard them.) The mixed vegetables were discharged into eight wooden boxes, four alternate ones for the open cooker, and the remainder for the pressure cooker. The contents of each box, about 150 lb., were mixed by hand as thoroughly as possible. Several small handfuls were then taken from the four boxes for the open cooker and combined to make about a 0.5 lb. sample. This was repeated. The same procedure was undertaken with the pressure-cooker vegetables. Determinations of vitamin C were then carried out, in duplicate, on 50 g. from each 0.5 lb. sample.

After sampling, the boxes of vegetables were added to the respective cooking waters, which were kept at about 85° and contained the meat just previously cooked. (Water boils at about 94° on the Witwatersrand.) During these additions the temperature fell to about 80°. The time taken from the beginning of mincing to the complete addition of vegetables was about 2 min. Salt was available for the Bantu, but neither salt nor any other chemical was added to the stew.

Cooking of the stew. For open cooking the time taken was 20–25 min. With the pressure cooker, it took 9 min. from the time of putting on to removing the lid, although the stew was maintained at a pressure of 12.5 lb. for 5 min. only. Each stew mass was rapidly stirred with a large soup ladle and two 0.5 lb. samples were taken from each cooker and analysed in duplicate for vitamin C.

Determination of vitamin C. Vitamin C was determined by the dichlorophenol-indophenol method as described by the Association of Vitamin Chemists (1947).

RESULTS

The results are given in Table 1.

The 575 lb. vegetables are mixed with 70 gal. (i.e. 700 lb.) water, to yield a total weight of 1275 lb. Hence, to obtain the vitamin C content of the stew before cooking, the vitamin C content of the mixed vegetables is multiplied by 575/1275, i.e. 0.45.

DISCUSSION

Considering the scale of the experiment, the high vitamin C content of the stew observed with both methods is satisfactory, though not surprising, for practical conditions are such as to favour a high retention of the vitamin. These are (a) the very fresh state of the vegetables; (b) the quick washing of the vegetables; (c) the rapid mincing and the short time elapsing before the vegetables are put into the cooking water; (d) the high initial temperature of the cooking water, 80–85°, resulting in the

Table 1. Vitamin C content of vegetables, raw and cooked on a large scale in open cookers and in pressure cookers

	1st day		2nd day		3rd day		4th day	
	Open cooker	Pressure cooker	Open cooker	Pressure cooker	Open cooker	Pressure cooker	Open cooker	Pressure cooker
Raw vegetables (mg./100 g.)								
	31.5	32.0	27.0	27.5	20.5	21.0	35.4	36.2
	32.5	28.0	21.5	20.5	24.0	24.2	25.8	25.0
	19.8	20.2	25.7	26.0	24.4	24.2	24.2	24.2
	20.6	26.3	29.0	29.3	25.3	24.2	27.2	27.0
	30.1	30.6	29.6	23.1	23.1	26.8	19.0	19.4
	31.1	—	23.2	20.0	—	—	19.6	21.9
		—	23.0	20.4	—	—	—	22.5
Mean*	27.6	26.7	24.5	26.6	24.2	26.0	20.8	21.8
Mean of stew before cooking† (mg./100 g.)	12.4	12.0	11.0	12.1	10.9	11.7	9.4	9.8
Stew after cooking (mg./100 g.)								
	11.7	11.9	10.0	10.0	9.7	9.8	8.2	8.1
	12.1	10.0	9.1	9.1	9.9	9.6	8.4	8.1
	11.1	10.1	8.6	8.8	9.8	9.2	8.5	8.2
	11.3	10.3	9.0	8.4	9.8	10.0	8.5	8.4
Mean	11.6	10.1	9.0	8.1	9.8	9.6	8.4	8.2
Retention of vitamin C (%)	94	84	82	67	90	82	90	84

* On two occasions determinations in duplicate were carried out on the individual vegetables minced separately. The values for sweet potatoes were 18.0, 18.8; 19.8, 20.6; mean 19.3 mg.; cabbage, 65.0, 67.2; 73.2, 70.0; mean 68.9 mg.; carrots, 4.8, 4.4; 5.4, 5.0; mean 4.9 mg.; leeks 25.5, 26.7; 26.6, 26.8; mean 26.4 mg./100 g. From these figures, calculation of the vitamin C in the vegetable mixture gives 28.4 mg./100 g., a value slightly higher than the mean of the eight values for mixed vegetables found above, i.e. 24.8 mg./100 g.

† In the present investigation the vitamin C content of the meat was not taken into account; moreover, meat was not included in the stew on which the vitamin determinations were carried out: the error thus introduced is very small.

immediate destruction of the relevant oxidases; and finally (e) the quick serving of the stew and its almost immediate consumption.

Ladles of about 500 g. capacity are used to serve the stew. Additional helpings are allowed, but they are not usually asked for because of the large helpings of other foods available. For each person, therefore, the stew provides 45–50 mg. vitamin C daily, well in excess of the 20–30 mg. recommended by the British Medical Association (1950).

The conclusion from this investigation is that for vitamin C retention there is no objection to the replacement of open cooking by pressure cooking. The main practical advantage is that there is an 80 % economy of steam. It might also be added, however, that the stew is, in the opinion of the consumers, more palatable when cooked by this method.

SUMMARY

1. There is a trend, in the compound kitchens of the gold mines of the Witwatersrand, to replace open by pressure cooking.
2. Since the vegetable stew is the chief, and sometimes the sole, source of vitamin C for the Bantu workers, among whom incidentally scurvy is very seldom observed, a comparison has been made between the vitamin C content of stew cooked on a very large scale by both methods.
3. Experiments have shown good retention of the vitamin with either method and a slightly better retention with open cooking, the mean values for tests carried out on four separate days being 89 and 79 % respectively.
4. The usual helping of stew, prepared by either method, provides daily 45–50 mg. vitamin C, an amount that may be considered adequate.
5. The conclusion reached is that for vitamin C retention there is no reason why pressure cooking should not be adopted.

The authors are grateful for the co-operation and assistance given by Dr K. Sartorius, Chief Medical Officer of Union Corporation, Dr P. R. Dickson, Medical Officer, and Mr N. J. Pretorius, Compound Manager.

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The Nutritive Value of Colostrum for the Calf

4. The Effect of Small Quantities of Colostral Whey, Dialysed Whey and 'Immune Lactoglobulins'

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In our experience (Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin & Lovell, 1949; Aschaffenburg, Bartlett, Kon, Walker, Briggs, Cotchin & Lovell, 1949) the factor most essential for the survival of the newborn calf is contained in the non-fatty fraction of colostrum, of which a very small quantity is sufficient to protect the calf against fatal scours. To define this factor more closely, the protective values of small quantities of colostral whey, dialysed whey and the 'immune lactoglobulins' (as defined by Smith, 1946) were compared with that of the separated colostrum from which these fractions were derived.

METHODS

Plan of experiment

The experiment was in two parts: one (autumn 1948) for the estimation of the relative effects on newborn calves of colostral whey, dialysed whey and separated colostrum; the other (spring 1950) for the comparison of the 'immune lactoglobulins' with separated colostrum. In both, the randomized block design was adopted, and one calf deprived of colostrum was included in each block. The layout was as shown in Table 1. In both experiments, eight blocks of Shorthorn bull calves were used. The calves were assigned to treatments and to pens, selected at random within each block.

Table 1

Autumn 1948		Spring 1950	
Treatment no.	Colostral fraction in first feed	Treatment no.	Colostral fraction in first feed
0	None	0	None
5	150 ml. separated colostrum	8	200 ml. separated colostrum
6	150 ml. whey	9	c. 14 g. 'immune lactoglobulins' in 155 ml. solution
7	200 ml. dialysed whey		

*Diets**Basic diet*

The calves were kept on the 'synthetic milk' (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949) for 3 weeks, the maximum daily allowance being 1 lb./10 lb. live weight, except when scouring occurred (see below).

Colostrals diets

The various colostrals fractions were given to the calves in their first meal within 12 hr. of birth.

Separated colostrum for autumn 1948 experiments. Four batches of colostrum obtained within 24 hr. of calving from Shorthorn cows were used. Each was warmed and passed twice through an ordinary cream separator. Part of each batch was stored at -25° , the remainder was used for the preparation of whey.

Whey. The separated colostrum was treated with rennet at 37° (1 ml. rennet extract/l.), and the whey clarified by filtration through a filter pad and through no. 3 Whatman filter-paper. Part of the whey was stored at -25° , the remainder was dialysed.

Dialysed whey. The whey was dialysed at 4° for 36–48 hr. in a rotating cellophane bag against continuously changing distilled water. Any protein precipitate found after dialysis was redispersed with the minimal quantity of sodium chloride. The product was stored at -25° .

The four batches of the different fractions were blended in equal volumes for feeding to the calves. The amount given to each calf in its initial feed contained approximately 13 g. of whey proteins.

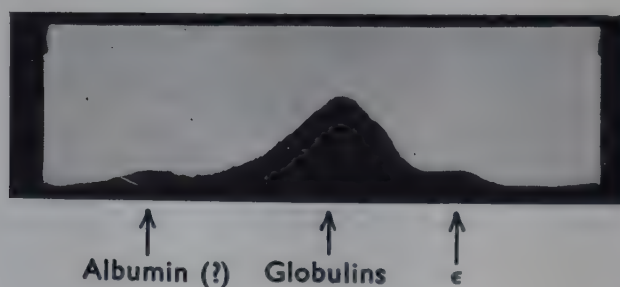


Fig. 1. Colostral globulins (batch no. 4). Descending boundary after electrophoresis for 165 min.

Separated colostrum for spring 1950 experiments. A similar procedure to that of the autumn 1948 experiment was adopted, and six batches instead of four were collected, this time whenever possible from the first milking only. If the quantity was insufficient, colostrum from the second milking was added. A portion of each batch was stored at -25° and 'immune lactoglobulins' were prepared from the rest.

'Immune lactoglobulins.' The method used for the preparation was essentially that of Smith (1946), which is based on the removal of casein followed by dialysis of the proteins precipitated by 0.4 saturation with ammonium sulphate. The great dilution required for the removal of the casein by acid precipitation was avoided by removing it by rennet (2 ml./l. at 37°). Firm curds were produced more readily by adding calcium-chloride solution (300 mg. Ca/l. colostrum). The pH of the clear, filtered whey

was adjusted to about 6.0 with acetic acid. The precipitated proteins were separated by centrifugation, dispersed in the least amount of distilled water, and dialysed at 4° as for the whey but for 3–4 days. Dialysis was stopped when SO₄ ions were no longer detected by reaction with barium chloride. At this stage part of the globulins had precipitated out and was redispersed by the addition of sodium chloride. The product contained about 10 % protein and was stored at –25°.

Six batches were prepared, and some details of the composition of those used are given in Table 2.

Table 2. *Composition of four batches of protein fractions of colostrum used in the spring 1950 experiments*

Batch no.	Protein (g./100 ml.)	Separated colostrum		'Immune lactoglobulins' as percentage of whey proteins
		Casein as percentage of total protein	Globulin as percentage of total protein	
2	12.3	29.2	51.5	72.7
4	14.8	38.4	43.3	70.2
5	12.7	23.7	61.3	80.4
6	15.0	33.4	46.1	69.3

For the feeding of the experimental calves, the batches of separated colostrum or of the 'immune lactoglobulins' were blended. Calves received either 200 ml. of blended separated colostrum containing 27.4 g. of protein, of which *c.* 50 % consisted of globulins, or 155 ml. of blended lactoglobulins with a protein content of *c.* 14 g.

Dr C. L. Hannay found that two of the batches of colostrum, nos. 4 and 5, had agglutinins against *Brucella abortus* to high titres, 1 : 2500 and 1 : 10,000 respectively. Similar titres were obtained when the 'immune lactoglobulins' were tested, and this suggests that the processes involved in the isolation of the globulins had not decreased the concentration of antibodies. It is assumed, but not yet proven, that any *Bacterium coli* antibodies present were also unimpaired.

A sample of the isolated whey globulins (batch no. 4) was equilibrated by dialysis against 0.055 M-sodium veronal-HCl buffer (Michaelis, 1930) of pH 8.42. After dilution with buffer to a protein content of 1 %, it was subjected by our colleague, Dr N. J. Berridge, to electrophoretic examination. Fig. 1 shows the conditions after migration for 165 min. at 10 mA. It indicates that the preparation consisted largely of globulins, though a faster moving component, probably albumin, was present. This was expected, as the centrifuged proteins precipitated by 0.4 saturation with ammonium sulphate had been dialysed without further purification.

Calves

Shorthorn bull calves were collected and managed as in earlier experiments (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949). On the arrival of a newborn calf a blood sample was taken and the globulin-turbidity test of Aschaffenburg (1949) was applied to the serum to verify that the calf had not suckled. Calves were rejected if the test was positive.

After the initial feed containing the selected fraction of colostrum all calves were fed three times daily for the first 10 days of life, and twice daily for the remainder of the period. When a calf scoured, one feed was omitted and 'synthetic milk' diluted with an equal amount of water was given at the next. The proportion of milk was gradually increased in subsequent meals until undiluted 'synthetic milk' was again given. This practice was repeated when scouring recurred. Records were kept as in the earlier experiment (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949).

RESULTS

Autumn 1948

The results for the eight blocks of Shorthorn bull calves (thirty-two animals) are summarized in Table 3.

Table 3. *Comparison of small amounts of whey and dialysed whey with separated colostrum*

Treatment no.	...	0	5	6	7
Diet given	...	No colostrum	150 ml. separated colostrum	150 ml. whey	200 ml. dialysed whey
Calves:					
No. used		8	8	8	8
No. died		8	3	3	3
Mean age of calves at death (days)*		4 ± 1	10 ± 2	11 ± 2	7 ± 1
Mean live-weight gain of sur- viving calves in 21 days (lb.)*		—	-2 ± 3.5	-9 ± 1.7	-9 ± 1.9
Mean no. of days on which surviving calves scoured*		—	3 ± 1	5 ± 1	6 ± 2

* Values with their standard errors of the mean. In calculating the standard errors the arrangement in blocks has been ignored.

All eight calves deprived of colostrum died, whereas only three died on each of the colostrum treatments. The χ^2 test, after adjustment for continuity, shows that the difference in mortality between treatment 0 and treatments 5, 6 or 7 is significant ($P < 0.05$).

The statistical significance of differences between treatments in the mean live-weight gains of calves, and in the mean number of days on which the surviving calves scoured on treatments 5-7 was determined by the *t* test ('Student', 1908, 1925), *P* values below 0.05 being regarded as significant. There were no significant differences between the colostrum treatments. To take out that part of the residual error due to differences between blocks, the missing plot technique of Yates (1933) was used, and values were calculated for the calves receiving the colostrum fractions that did not survive the experimental period. Analysis of covariance of live-weight gain on birth weight and analysis of variance of the number of days on which scouring occurred (*x*), with the values transformed $\sqrt{(x + \frac{1}{2})}$, still gave no significant differences between the colostrum treatments.

Spring 1950

The results for the eight blocks of Shorthorn bull calves (twenty-four animals) are summarized in Table 4.

Table 4. *Comparison of small amounts of 'immune lactoglobulins' with separated colostrum*

Treatment no. ...	0	8	9
Diet given ...	No colostrum	200 ml. separated colostrum	c. 14 g.* 'immune lactoglobulins'
Calves:			
No. used	8	8	8
No. died	8	3	3
Mean age of calves at death (days)†	6±2	9±2	7±1
Mean live-weight gain of surviving calves in 21 days (lb.)†	—	-3±2.6	+1±1.5
Mean no. of days on which surviving calves scoured†	—	9±2	7±1

* Contained in 155 ml. of solution.

† Values with their standard errors of the mean. In calculating the standard errors the arrangement in blocks has been ignored.

Again all eight calves deprived of colostrum died, whereas only three died on each of the colostrum treatments.

Statistical analysis on the lines already described again gave a significant difference ($P < 0.05$) between the mortality rate of calves on treatment 0 and that of calves on treatments 8 or 9, but there were no significant differences between the two colostrum treatments for the mean live-weight gain and the mean number of days on which the calves scoured.

Autopsy findings

A summary of the autopsy findings is given in Table 5. Of the thirty-one calves that died, twenty-two died from a *Bact. coli* septicaemia, and eight from *Bact. coli* peritonitis and pleurisy. These are manifestations of the disease classified as colibacillosis

Table 5. *Summary of autopsy findings*

Treatment no. ...	0	5	6	7	0	8	9
Diet given ...	No colostrum	Separated colostrum	Whey	Dialysed whey	No colostrum	Separated colostrum	'Immune lactoglobulins'
Total no. of calves taken for autopsy	8	3	3	3	8	3	3
Findings at autopsy:							
Colibacillosis:							
Fatal scours including septicaemia	8	3	2	2	5	2	—
<i>Bact. coli</i> peritonitis and pleurisy	—	—	—	1	3	1	3
<i>Corynebacterium pyogenes</i> pneumonia	—	—	1	—	—	—	—

or 'fatal scours'. In the peritonitis and pleurisy cases, *Bact. coli* was invariably isolated from the heart blood and bone marrow as well as from elsewhere.

DISCUSSION

These experiments, together with previous work (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949; Aschaffenburg, Bartlett, Kon, Walker *et al.* 1949), have shown that under our conditions, the survival of the newborn calf is dependent on a factor contained in the globulin fraction of colostrum. Comparison of mortality rates indicates that in the present experiments the small quantities of the colostrum fractions gave less protection than the small quantities of separated colostrum given in an earlier experiment (Aschaffenburg, Bartlett, Kon, Walker *et al.* 1949). Neither prevented scouring, and with both weight gains were subnormal. The reasons for the subnormal growth of calves given small amounts of the protective fractions of colostrum will be studied in future work.

SUMMARY

1. Shorthorn bull calves, grouped in two experiments, each of eight blocks, were given separated colostrum, or fractions of it, in the first 12 hr. of life, followed for 3 weeks by a standard diet based on dried skim milk. One calf in each block was deprived of colostrum.
2. All calves deprived of colostrum died. Twenty-five of forty calves given small quantities of separated colostrum, whey, dialysed whey or 'immune lactoglobulins' survived. These diets did not, however, prevent the calves from scouring and the calves did not gain weight normally.
3. There was a significant difference between the mortality rate of calves deprived of colostrum and that of calves receiving the colostrum fractions. There was no significant difference between the performances of the surviving calves that received the colostrum fractions.

The work here reported was done under a special grant from the Agricultural Research Council. We wish to thank Dr C. L. Hannay and Dr N. J. Berridge for their help. Our thanks are also due to Mr H. J. Sears for his assistance with the experimental animals and to the farmers in the neighbourhood who supplied the experimental calves.

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Dietary Surveys: Variation in the Weekly Intake of Nutrients

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Much effort has been expended in recent years in assessing the adequacy of diets by measuring food consumption. The principle is to determine the intake of nutrients and to compare this with standards of requirements: an intake lower than the requirement is then taken to indicate an inadequate diet. The limitations in the use of these standards have been discussed elsewhere (Yudkin, 1948). The present paper deals with some of the limitations in the methods of determining dietary intakes, and more particularly with the week-to-week variation in intakes.

The practical difficulties involved have led to the adoption of many different methods, which vary greatly in complexity. In many circumstances, it is quite impossible to use the more complex methods, so that the simpler methods are used perforce and accuracy is sacrificed to expediency. The extent of the errors likely to be introduced has recently been the subject of investigation, so that the limitations of the various methods are now becoming known (see, for example, Bransby, Daubney & King, 1948*a, b*).

Whatever method is chosen, it remains necessary to decide for how long the measurement of intake should be made. Because of the laborious nature of even the simpler methods of conducting surveys, this question resolves itself in practice into deciding the minimal time which reasonably reflects the normal pattern of food consumption. It is recognized that the amounts of different foods consumed vary from day to day and that, in most western dietaries, this variation may be quite large (cf. Wait & Roberts, 1932; McKay & Patton, 1935). Most workers agree that, in western countries, it is necessary to measure the intake for a week. More important, they also agree that such a period is sufficient to give a reasonably accurate assessment of the amounts and sorts of foods habitually consumed by individuals (cf. Food and Agriculture Organization of the United Nations, 1949). But there is relatively little published on this point. Widdowson & McCance (1945) and Widdowson (1947) studied the diets of 'a number of children' for periods of 4 consecutive weeks. From these results and from similar results of Boulton (1945), Widdowson (1947, p. 73) concludes that, at least for calories, a week's study gives a fair assessment of a person's intake. On the other hand, McHenry, Ferguson & Gurland (1945) reported great variation in weekly intakes measured for the 1st week of each month for a year. Their results led them to decide that 'dietary surveys for a period of one week should not be used to ascertain the extent of deficiencies in persons having... a free-will choice of foods'.

Further data on the adequacy of weekly studies of intake are clearly desirable, and this paper reports an investigation designed to provide them.

EXPERIMENTAL

Details of subjects. It is usual for the postgraduate dietetic students at this College to determine their own dietary intakes for 1 week. During the session 1948-9, the five students of that year and one of the members of the staff were asked to keep records of their dietary intakes for 4 consecutive weeks. Two of the students measured their intakes for a further week after a short interval. All of the subjects were women. One of them (subject no. 6) was in the 5th month of her first pregnancy. Table 1 shows the periods during which the food consumption was measured and also gives some personal data of the subjects. None of them had any illnesses during these periods; it might also be stated at once that there was no obvious relationship between food consumption and menstruation.

Table 1. *Data on the six women studied and time of the study*

Subject no.	Period of study	Age (years)	Weight (lb.)	Height (in.)
1	15 Nov.-12 Dec. 1948 30 Jan.-5 Feb. 1949	21	131	63
2	14 Nov.-11 Dec. 1948 30 Jan.-5 Feb. 1949	20	121	67
3	16 Nov.-13 Dec. 1948	21	146	70
4	17 Nov.-14 Dec. 1948	20	159	66½
5	17 Nov.-14 Dec. 1948	26	108	61½
6	21 Apr.-19 May 1949	34	160	67½

Food consumed and calculation of nutrients. All of the subjects ate 'elevenses', lunch and tea at the College from Monday to Friday, and the remaining meals at their homes or lodgings or a students' hostel. It was thus possible to ascertain the composition of most made-up dishes and calculate the amounts of nutrients therein from the ingredients. The food consumed was weighed by each subject herself.

Three of the subjects took daily supplementary amounts of nutrients as follows. Subject no. 2: vitamin A 500 i.u., vitamin D 1400 i.u., calcium 325 mg. Subject no. 3: vitamin A 4500 i.u., vitamin B₁ 0.15 mg., riboflavin 0.05 mg., vitamin C 10 mg., vitamin D 600 i.u. Subject no. 6: vitamin A 4000 i.u., vitamin D 800 i.u., calcium 750 mg.

The nutrients in the diets were calculated by reference to food tables. Almost all the values were taken from 'Nutritive Values of War-time Foods' (Accessory Food Factors Committee, 1945), with some additional and revised values kindly supplied by the Ministry of Food. Occasionally, it was necessary to refer to the tables prepared by Platt (1945).

Since it is now agreed—and was readily confirmed in this study—that the intake varies greatly from day to day, and since the purpose of this work was to determine the weekly variation in intake, all the results have been calculated as the average daily intake for each week. These averages, and the average for the whole 4-weekly period, are given graphically for calories and for each nutrient; where appropriate, the graphs

also indicate the recommended allowances suggested by the British Medical Association (1950) and by the (U.S.A.) National Research Council (1948). These will be referred to as the allowances of the B.M.A. and N.R.C.

Since subject no. 6 was about half-way through her pregnancy during the measurement of her intake, her allowances have been taken as the mean of those for the first half and for the second half of pregnancy.

In order to give a truer picture of the nutrients contained in the diets as consumed, the supplementary nutrients, taken by subjects nos. 2, 3 and 6, have not been included in the results given nor considered in the discussion thereon.

RESULTS

Variation in weekly intake

The variations in intake between subjects, and the variations from week to week in the same subjects, are given in Figs. 1-4. It will be seen that the extent of the weekly variation differs from subject to subject and also from nutrient to nutrient. It is least with calories, where the difference between the highest and lowest intake of an individual is from 2 to 68 %; it is most with vitamin D, where the difference is from 250 to 650 %.

Some special points about individual nutrients are worth noting.

Calories. The recommended allowances of calories for the five students have been taken as those for 'light work and travelling' (2250 Cal., B.M.A.) or for 'moderately active women' (2400 Cal., N.R.C.). Throughout the 4 weeks, the intake of calories by subject no. 5 was appreciably lower than the recommended allowances and those by subjects nos. 2 and 4 slightly lower.

The contributions made by protein, fat and carbohydrates to the total caloric intake are shown in Table 2.

Table 2. *Calories supplied by protein, fat and carbohydrate in the diets of the six subjects averaged over 4 weeks*

Subject no.	Percentage of calories supplied from		
	Protein	Fat	Carbohydrate
1	14	39	47
2	12	40	48
3	14	37	49
4	15	35	50
5	17	40	43
6	14	35	51

Protein. Since it is held that, for reasons concerned more with the presence of associated factors than with its content of essential amino-acids, protein from animal sources is nutritionally superior to protein from vegetable sources, a summary is given of the proportion of the total protein derived from animal foods (Table 3). In addition, a calculation has been made of the proportion of the calories supplied by the protein, since it has been suggested that an adequate intake of protein is one which in normal

adults supplies 11 % of the calories or which, in pregnant women, supplies 14 % of the calories (British Medical Association, 1950).

In four of the five students, the proportion supplied from animal foods was between

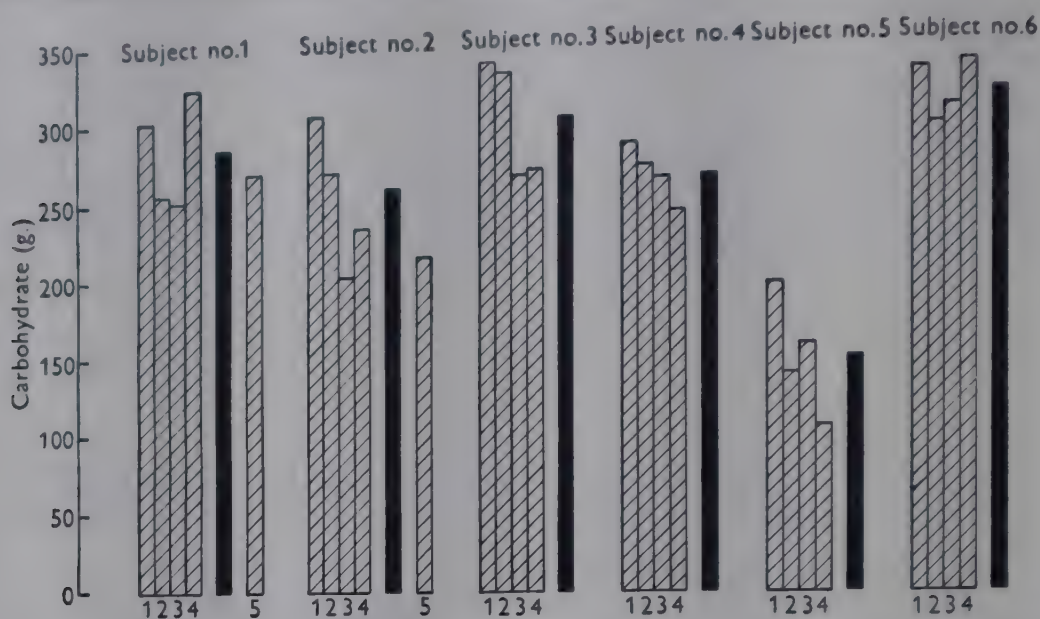


Fig. 1. Variation in weekly intake of carbohydrate, fat, protein and calories of the six subjects. Hatched columns show average daily intake in each of the 4 (or 5) weeks of study. Solid column gives mean daily intake over 4 consecutive weeks. Dotted lines indicate recommended allowance of the (U.S.A.) National Research Council (1948) (N.R.C.) and British Medical Association (1950) (B.M.A.).

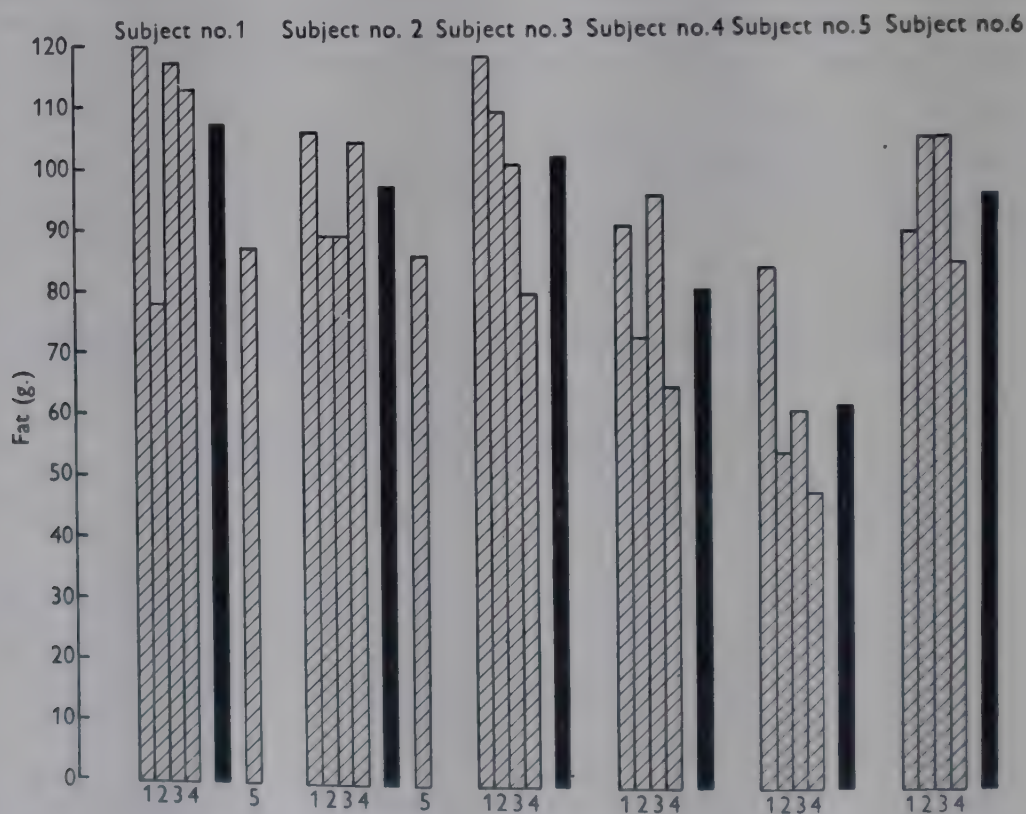


Fig. 1 (cont.)

52 and 56 %. The fifth took 70 % of her protein in animal foods. Much of this came from milk; her average consumption of this for the 4 weeks was 0.38 l. daily, whereas the other four students took an average of 0.10–0.25 l. daily. The pregnant subject

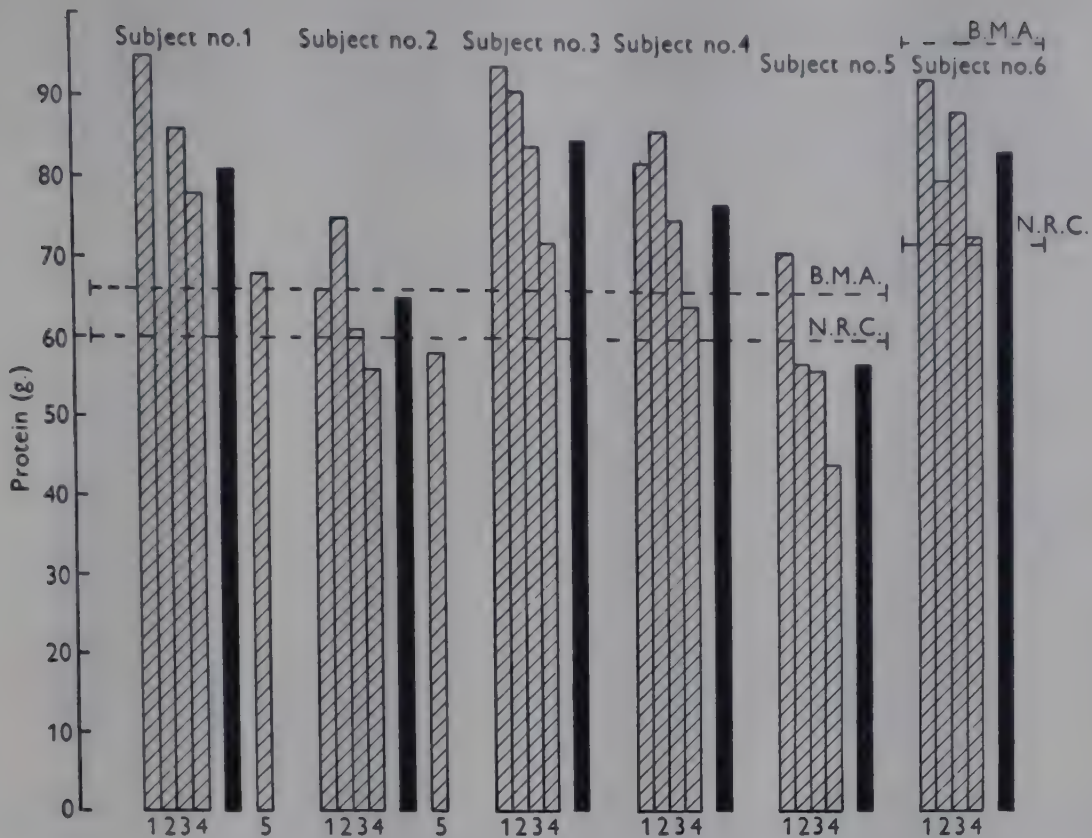


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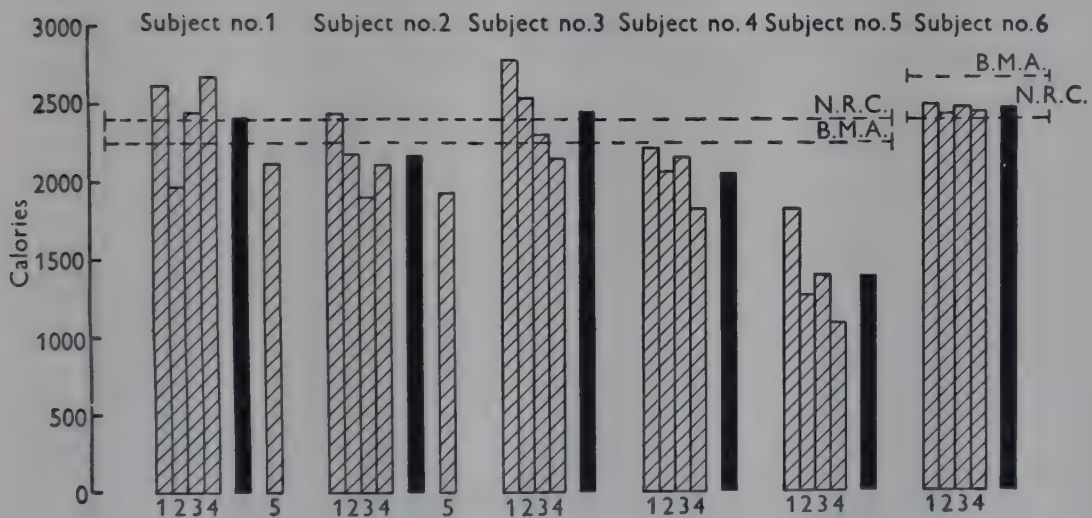


Fig. 1 (cont.)

Table 3. Average daily protein intake of the six subjects over 4 weeks, proportion of animal protein and proportion of calories supplied by protein

Subject no.	Average daily total protein intake (g.)	Percentage of total protein derived from animal foods	Percentage of total calories derived from protein
1	81	53	13.8
2	65	52	12.3
3	84	56	14.2
4	77	55	15.3
5	57	70	16.8
6	84	66	13.9

no. 6 also had a high proportion of animal protein (66 %) in her diet; her milk consumption was higher than that of any other subject, 0.49 l. daily. The proportion of calories supplied by protein was near enough to the suggested allowances of 14 % for the pregnant subject; for the other subjects, it was well above the suggested 11 % (12.3–16.8 %).

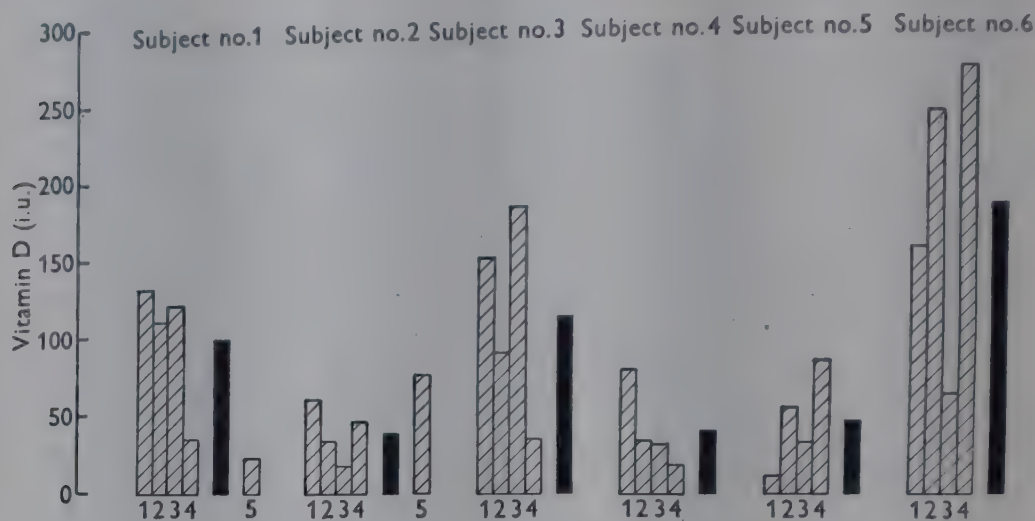


Fig. 2. Variation in weekly intake of vitamin D, vitamin A and carotene, iron, and calcium of the six subjects. The contribution of carotene was calculated on the assumption that 3 i.u. carotene are equal to 1 i.u. preformed vitamin A. For further information see Fig. 1.

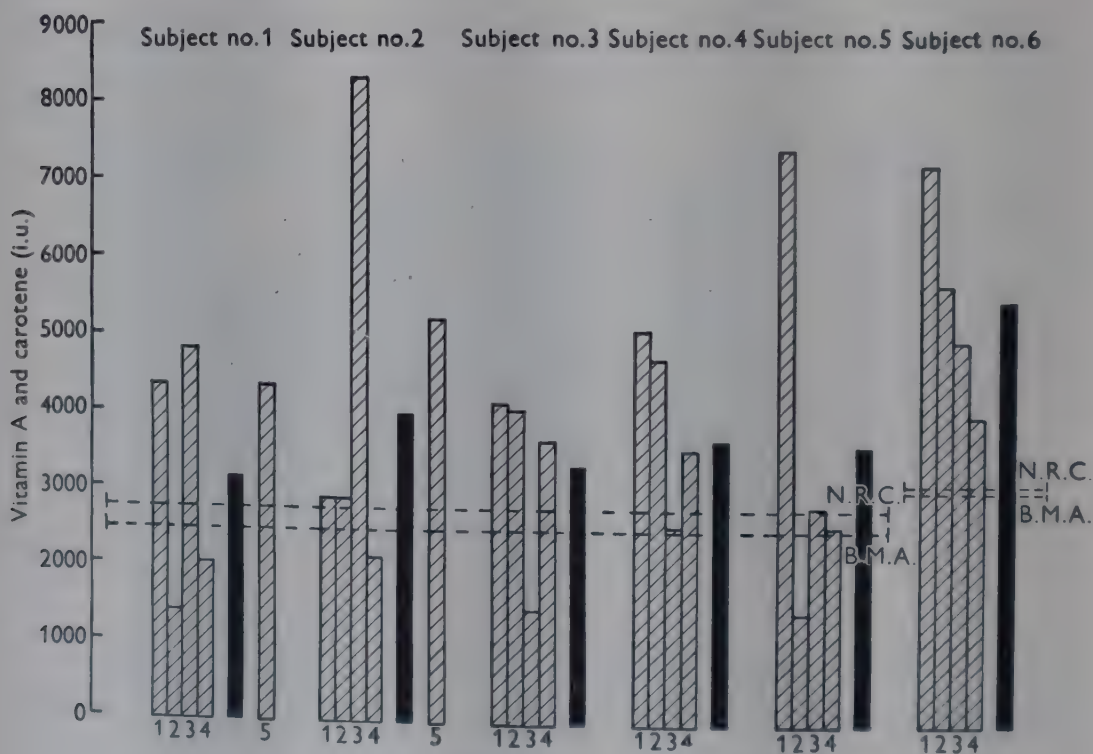


Fig. 2 (cont.)

Calcium. As with protein, the intake of calcium of subject no. 5, in spite of her low consumption of food in general, was as good as that of the other students because of her high intake of milk. Again, the highest intake of calcium was that of subject no. 6 whose diet contained the highest amounts of milk.

No allowance has been made for calcium supplied from tap water, which in London

would increase the intake by about 0.1 g. (Widdowson & McCance, 1943). This would bring the average 4-weekly intake of the five students well above the B.M.A. standard but still below the N.R.C. standard.

Vitamin A. It is now known that there is appreciable variation in the vitamin A activity of carotene, depending both on the subject consuming it and on the food

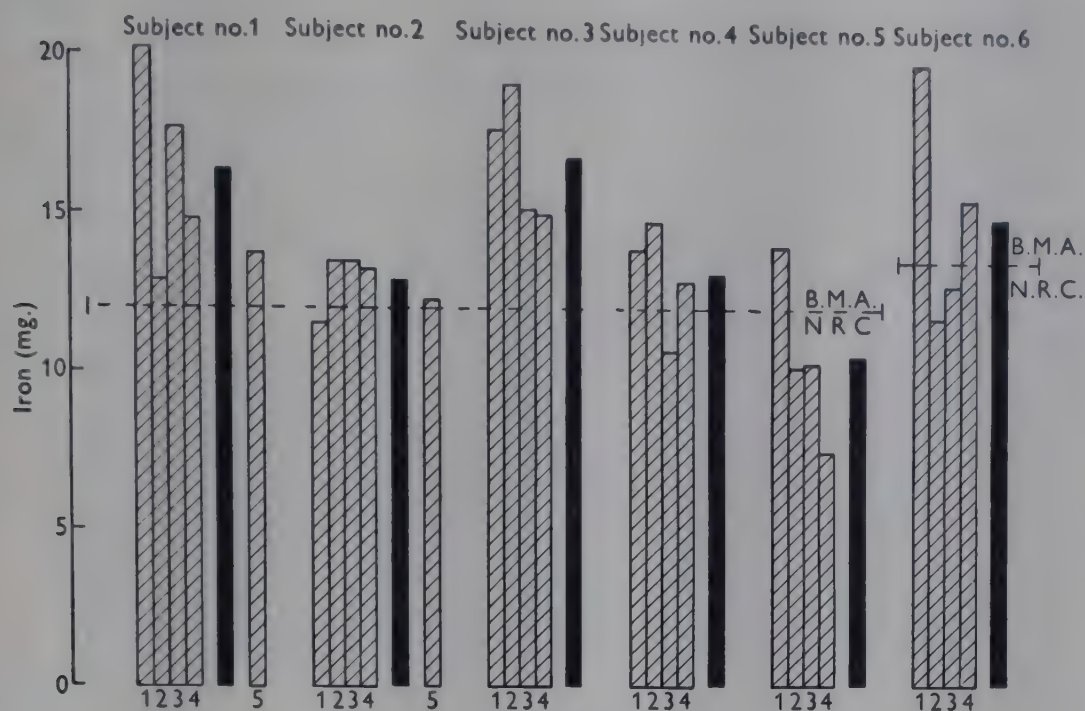


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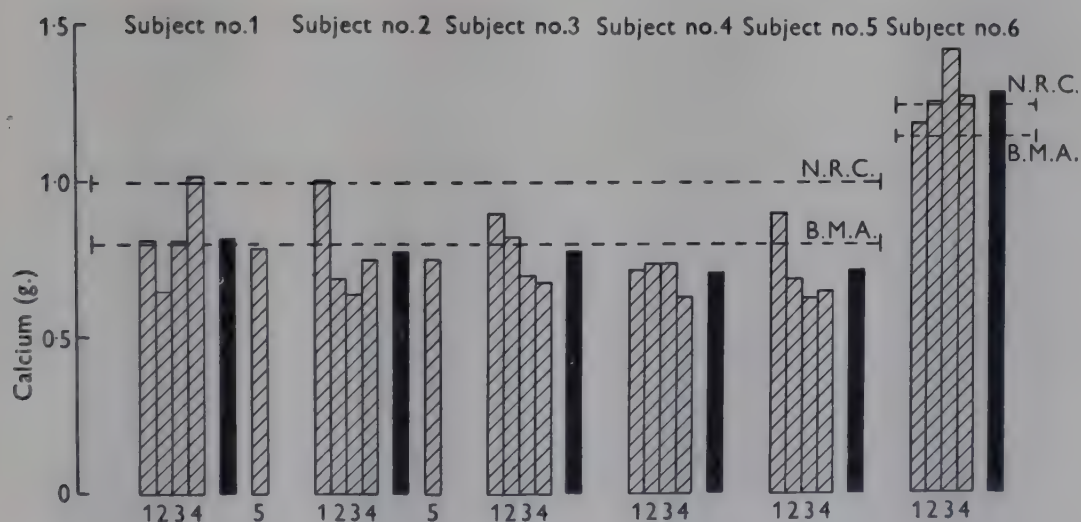


Fig. 2 (cont.)

from which it comes. A reasonable estimate, however, is given if it is assumed that 3 i.u. carotene are equal in potency to 1 i.u. of preformed vitamin A. This assumption has been made in the calculations herein reported.

The chief sources of vitamin A in the weeks of high consumption were liver and carrots. For example, one helping of 152 g. liver in the 3rd week gave subject no. 2 about 33,000 i.u. vitamin A, an average of about 4700 i.u. daily. Four helpings of 287 g. carrots in the 2nd week gave subject no. 4 about 60,000 i.u. carotene, equivalent

to 20,000 i.u. vitamin A in a week or an average of about 3000 i.u. vitamin A daily. Without these two rich sources of the preformed vitamin or of carotene, the intake would have varied much less from week to week (Table 4).

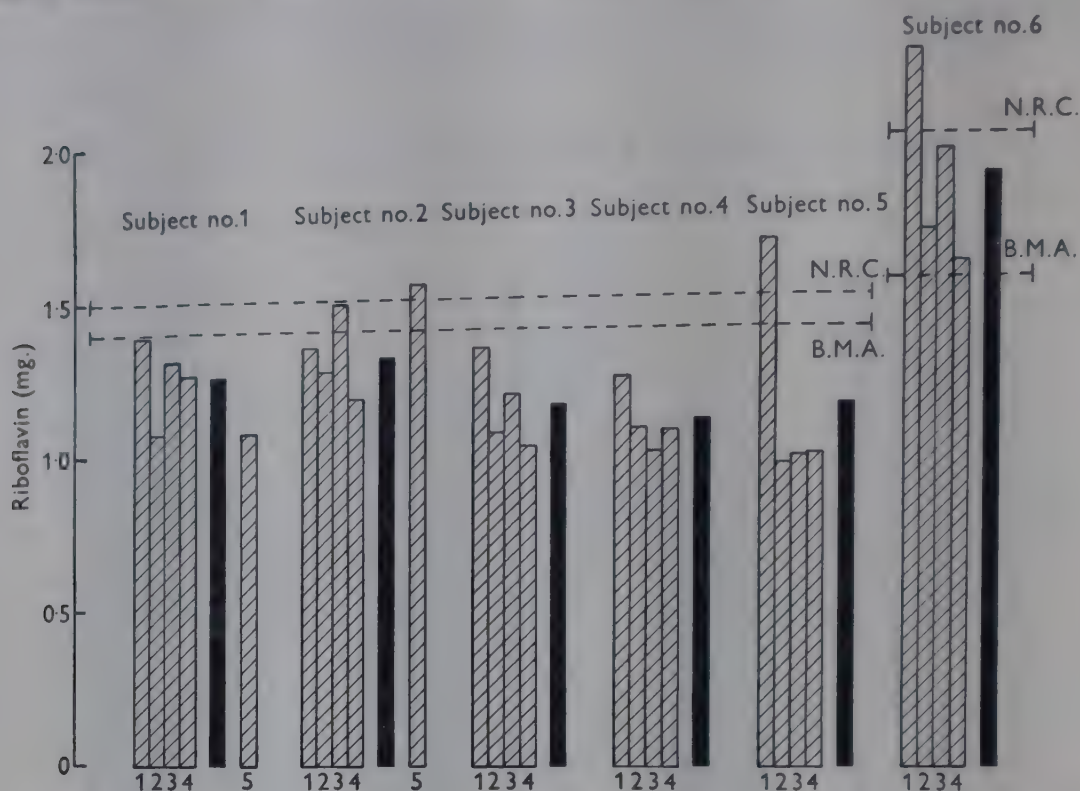


Fig. 3. Variation in weekly intake of riboflavin, nicotinic acid, vitamin C and vitamin B₁ of the six subjects. For further information see Fig. 1.

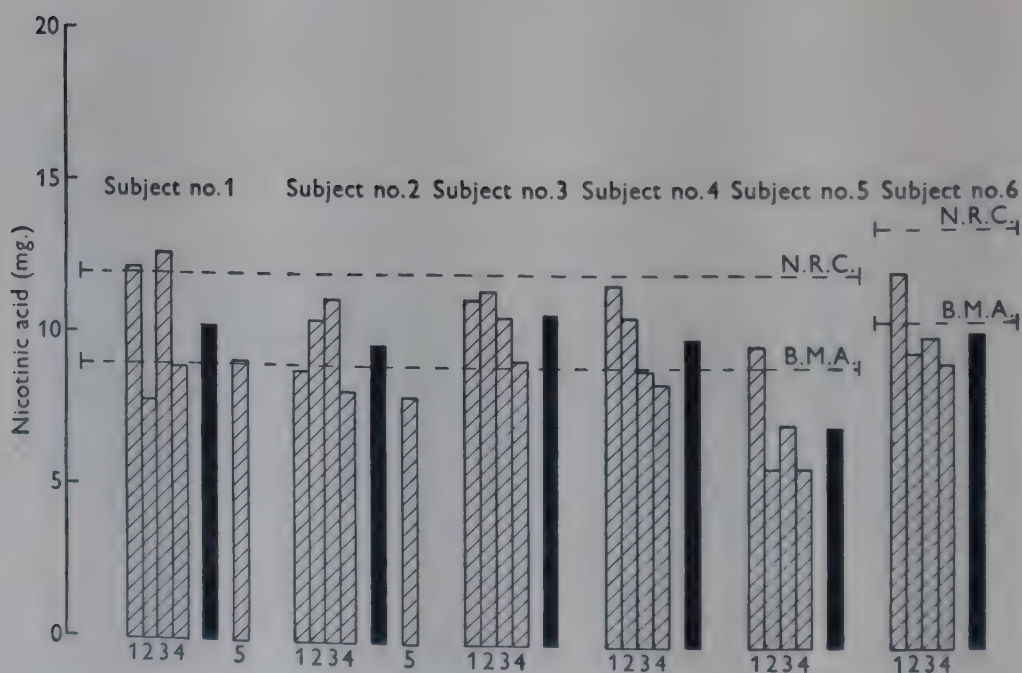


Fig. 3 (cont.)

The average mixed western diet is said to contain two-thirds of its vitamin A as carotene. On the assumption that carotene has one-third the potency of the preformed vitamin, this would mean that it constitutes 40 % of the total dietary vitamin A. The actual value varies from 30 to 50 % (Table 5).

Riboflavin. The average daily intake of subject no. 6, 1.88 mg., was higher than that of any of the other subjects and, like her intake of calcium, was to a large extent due

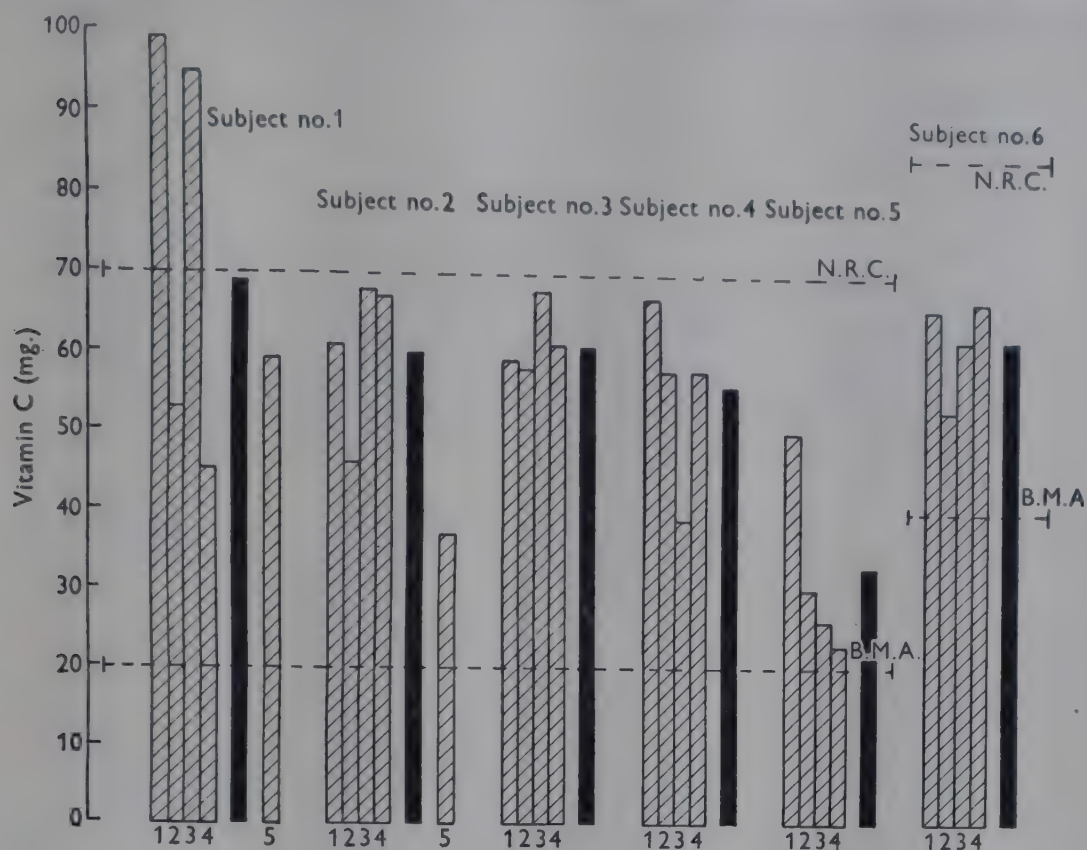


Fig. 3 (cont.)

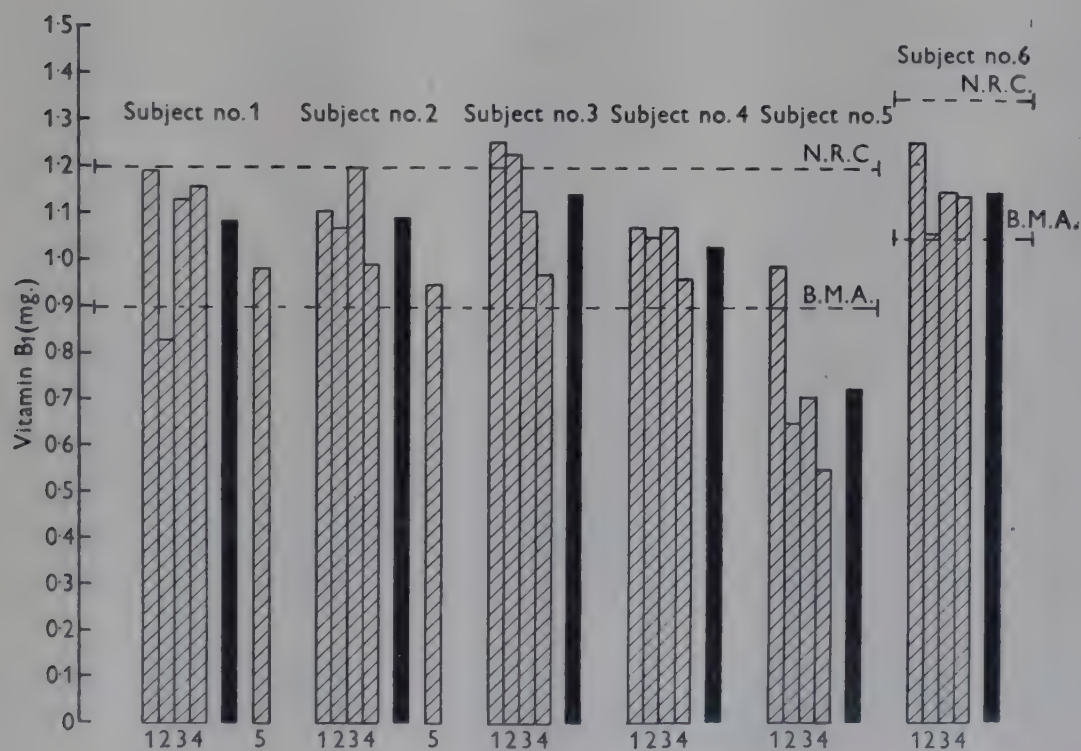


Fig. 3 (cont.)

to her high consumption of milk. This alone supplied an average of nearly 1 mg. of riboflavin daily.

Vitamin C. The values given for vitamin C allow for losses in cooking. Owing,

however, to the great differences that may occur in such losses, due, for example, to differences in time of cooking, amount of water used, and the time for which vegetables may be kept on the hot plate, the values for vitamin C are likely to be less accurate

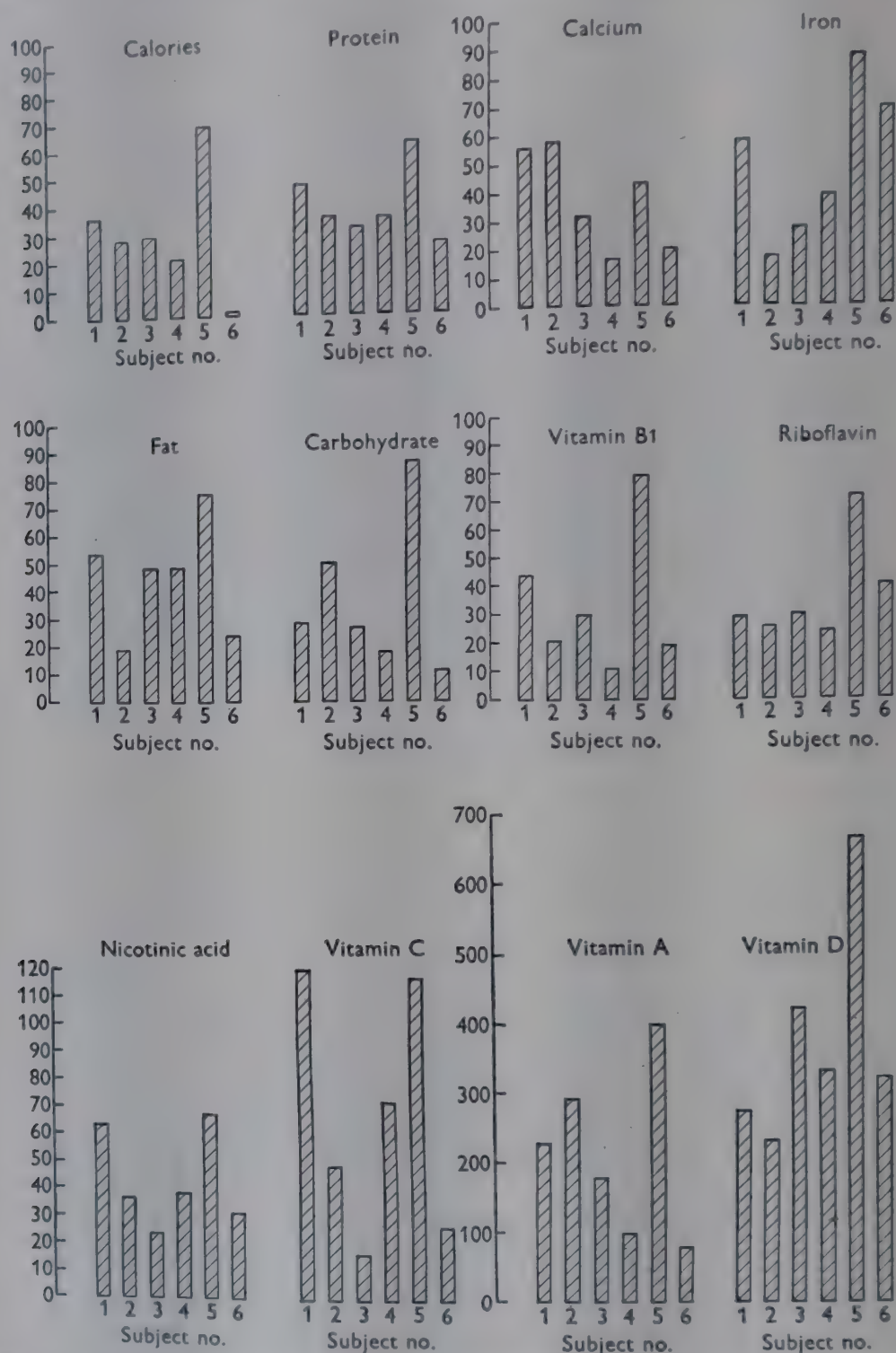


Fig. 4. Difference between highest and lowest weekly intakes of calories, protein, calcium, iron, fat, carbohydrate, vitamin B₁, riboflavin, nicotinic acid and vitamins C, A and D of the six subjects. Figures give difference as percentage of lowest intake.

than those for other nutrients. Nevertheless, the relative values in the different weekly measurements are probably reliable.

Vitamin D. The weekly variation with each subject is due, like that of vitamin A, to the presence or absence in the weekly diet of a potent source of the vitamin. With

Table 4. *Average daily intake (i.u.) of vitamin A supplied by liver, carrots and other foods in the diets of the six subjects*

Subject no.	Source of vitamin A*	Week				
		1	2	3	4	5
1	Liver	1000	0	900	0	0
	Carrots	1080	0	1000	0	3050
	Other foods	2280	1470	2930	2040	1310
2	Liver	0	0	4640	0	3010
	Carrots	260	790	1000	0	0
	Other foods	2640	2110	2680	2120	2190
3	Liver	950	0	0	1250	—
	Carrots	1240	1860	150	1320	—
	Other foods	1950	2190	1330	1090	—
4	Liver	1560	0	0	1460	—
	Carrots	2170	2860	1040	520	—
	Other foods	1360	1850	1510	1570	—
5	Liver	3290	0	0	780	—
	Carrots	1860	110	910	350	—
	Other foods	2230	1370	1890	1430	—
6	Liver	4240	0	0	0	—
	Carrots	510	2670	1810	0	—
	Other foods	2420	2980	3130	3980	—

* Calculated for carotene on the assumption that 3 i.u. carotene are equal to 1 i.u. preformed vitamin A.

Table 5. *Average daily intake (i.u.) of vitamin A and carotene by the six subjects**

Subject no.		Week				Mean of 4 weeks	Week 5
		1	2	3	4		
1	Vitamin A	2120	1020	2740	1540	1850	980
	Carotene	2240	450	2090	500	1320	3380
	Total	4360	1470	4830	2040	3170	4360
2	Vitamin A	1840	1240	6590	1830	2770	4880
	Carotene	1060	1660	1730	290	1200	320
	Total	2900	2900	8320	2120	3970	5200
3	Vitamin A	2390	2000	1140	2140	1920	—
	Carotene	1750	2050	340	1520	1420	—
	Total	4140	4050	1480	3660	3340	—
4	Vitamin A	2540	1250	890	2470	1820	—
	Carotene	2550	3460	1660	1080	1840	—
	Total	5090	4710	2550	3550	3660	—
5	Vitamin A	4830	1140	1280	1920	2330	—
	Carotene	2550	340	1520	640	1250	—
	Total	7380	1480	2800	2560	3580	—
6	Vitamin A	6230	2460	2030	2230	3240	—
	Carotene	940	3190	2910	1750	2200	—
	Total	7170	5650	4940	3980	5440	—

* See footnote to Table 4.

vitamin D, this potent source is fatty fish such as herrings, salmon or sardines. If the contribution of vitamin D from this source is deducted, the weekly intake varies much less (Table 6). The highest weekly average of each subject is then between 43 and

Table 6. *Average daily intake (i.u.) of vitamin D by the six subjects during 4 weeks*

Source	Subject no.					
	1	2	3	4	5	6
Fish	63	0	74	13	34	111
Margarine	15	16	11	9	0.5	45
Other foods	21	23	27	18	12	27
Total	99	39	112	40	46.5	183

233 % greater than the lowest, compared with between 233 and 664 % when the vitamin D from fish is included. The difference between the subjects, from 12 to 72 i.u. daily over the 4 weeks, is now chiefly due to differences in the amount of margarine eaten. Over the 4-weekly period, margarine gave an average daily intake between 45 i.u. (subject no. 6) and 0.5 i.u. (subject no. 5); excluding fish and margarine, the diet supplied between 12 and 27 i.u. daily.

DISCUSSION

Accuracy of determination of nutrients

Satisfactory assessment of the nutrients usually consumed by a person depends both on the accuracy of the determination of intakes and on a period of observation necessary to give a representative picture of the dietary pattern. In methods involving the use of food tables, there are two possible sources of error: firstly, errors may occur in the measurement of the amount of foods consumed and, secondly, there may be differences between the actual intake of nutrients and that calculated from food tables. It is possible to imagine that, unless these errors give results that are consistently too high or too low, the two factors of accuracy of method and period of observation might tend to cancel out, and an intake measured over a longer period would not only give a better assessment of the food normally consumed but more accurate values for the nutrients in the foods.

The extent to which the use of food tables gives results different from those of actual analysis has been investigated by several workers. Widdowson & McCance (1943) concluded that the results obtained by weighing intakes and calculating from food tables were in good agreement with those obtained by analysis for protein, fat, potassium, magnesium and phosphorus, but too low for calcium and iron. Both of the latter are added to the food during cooking, the former from hard water and the latter from the use of iron cooking utensils. Still more calcium can be consumed in the water itself. The results of Widdowson & McCance would imply that food tables can be legitimately used to assess nutrient intakes, provided it is recognized that the values calculated for calcium and iron are minimal.

More recently, Bransby *et al.* (1948 *a, b*) found that weighing the food and calcula-

tion from food tables gave average values for groups of subjects in quite good agreement with chemical analyses for protein, fat, carbohydrate and calcium, but too low for iron. For individual diets, however, they found appreciable differences between the two methods. These workers studied intakes for a period of 3 days; the fact that the two methods gave better agreement for groups of persons than for individuals indicates that the errors involved in the method of weighing and using food tables tend to cancel out. This supports the suggestion that accuracy is increased for individual intakes when more results are obtained in a more prolonged period of study.

The question then arises as to how much the differences in nutrient intakes revealed in the present study are due to inaccuracies of method. Sources of error in determining food consumption were probably as low as can be achieved. All of the subjects, who weighed their own food, were graduates in science and specializing in dietetics; they were, therefore, not only used to accurate weighing but appreciated the need for this and for accurate recording. Any questions as to method that arose during the survey were answered at once. In addition, since they were able to obtain the detailed recipes for most made-up dishes, it was possible to calculate the nutrients in these more accurately than from tables for 'composite dishes'.

The second source of error, the difference in composition between food consumed and food listed in the tables, could only have been checked by chemical analyses. The results of other workers (quoted above) suggest that the present results, which refer to individual diets, almost certainly do not give an exact record of the quantities of nutrients consumed. It is, moreover, likely that the error is greatest where one nutrient is supplied in large amount by one or two potent sources consumed irregularly, for example vitamin A from liver. Here the variation in composition of different samples of liver is wide and the figure in the food tables is an average value, so that it is quite possible that the amount of vitamin A actually consumed was appreciably different.

These comments are concerned chiefly with the absolute values of nutrients determined from food tables. They would apply less to relative values, for example the comparison of intakes in different weeks by the same or different persons. It is reasonable to assume that the differences (either between subjects or periods) revealed in this study are real within moderate limits. The subjects consumed much of their food in the College, and their diets, over the 4 consecutive weeks studied, were not influenced by seasonal variation in the composition of foods.

In the discussion which follows, therefore, it will be assumed that, whereas the absolute intakes of nutrients may not be exact, the relative intakes as between subjects, and as between different periods for the same subject, are reasonably correct.

Variations in weekly intake

Calories. One of the interesting features of the intake of calories is the difference in the extent of variation between the subjects. The intake of subject no. 6 was almost constant, whereas that of subject no. 5 in the 1st week was 68 % more than that in the last week. One would like to know whether the degree of variation is the same for any one subject, or whether it changes; in a different study, for example, would it be found

that subject no. 5 again showed a variation of the same degree, or might she this time have a more constant intake, whereas subject no. 6 varied more?

That there is such a large difference in the intake of calories with some subjects is, of course, most important. It will be recalled that, from studies of a week's intakes in each of 12 months, McHenry *et al.* (1945) decided that weekly intakes are unrepresentative. In the only subject whose intakes are quoted in their paper, the daily average was 1862 Cal. in one week and 2312 Cal. (24 % greater) in another. On the other hand, Widdowson & McCance (1945) and Widdowson (1947, p. 73) concluded that the determination of a week's intake of calories was sufficiently accurate for practical purposes. Yet a closer examination of Boulton's (1945) figures, quoted by Widdowson in support of this conclusion, shows that, of eight children studied for 4 weeks, the calories in the week of highest consumption were from 9 to 34 % more than in the week of lowest consumption. In three of these eight children the difference was at least 28 %. It is difficult to reconcile these results with Widdowson's statement that 'a fair estimate of a person's intake (of calories) is obtained from a study lasting for 1 week'.

The fact that one person's intake of calories may be greater by 50 % or more in one week than in another makes it easier to understand one of the most puzzling results of Widdowson's study of individual children's diets. In each of the age groups she studied, she found one child who had twice as many calories as another. The explanation for this which first comes to mind is a difference in requirements due to a difference in size (and hence in basal metabolism), a difference in activity and a difference in rate of growth. To these must be added possible errors in technique. Although the greatest care was taken to instruct the child or mother in weighing and recording food intakes, it is possible that the accuracy of the measurements was less than, say, the accuracy of the subjects of the present survey. Again, there is the error already discussed derived from the use of food tables. Nevertheless, Widdowson's results were still difficult to understand, for it seemed unlikely that all of these factors could account for one child consuming twice as much food as another of the same age.

Now, however, that we have seen how great can be the variation in one subject from week to week, it is quite possible to explain most, if not all, of this difference. Of the subjects of the present study, subjects nos. 1 and 3 had much the same average intake of calories over the whole 4-week period; yet even if they never varied more than they did during this period, subject no. 1 might easily consume 25 % more calories than subject no. 3 in one week and 30 % fewer calories in another week. Again, subject no. 5, whose average intake of calories in 4 weeks was indeed lower than that of subject no. 4, might nevertheless in one week have had the same intake and in another week less than half that of subject no. 4. In general, therefore, it seems justifiable to conclude that chance variation alone can account for one person's intake of calories for a week being 50 % or more greater than another person's intake. Such differences, together with differences in requirements and unavoidable errors due to technique, would together go a long way to explain how one child could in a week eat twice as much food as another child of the same age.

Protein, fat and carbohydrate. The weekly variation of these dietary components was

similar, and one may conclude that, in one week, a person might well consume 50 % more of each of them than in another week. Since there are no recommended allowances for carbohydrate and fat, variation in intake of these components need not be discussed. The situation, however, is different in respect of protein, for which standards of requirements have been laid down. A variation in weekly intake of the order found in these subjects may well mean that a diet studied for only 1 week might suggest a deficiency of protein in persons who usually consume adequate amounts. This applied, for example, to subject no. 2, whose average daily intake was 56 g. in 1 week but 65 g. over 4 weeks.

Calcium and iron. The actual amounts of these elements consumed by the subjects are likely, as we have seen, to be higher than the calculated amounts. A moderate increase in calcium will occur because of its presence in water, and an appreciable increase in iron might occur because of its being added to the diet from iron utensils. It is not possible to know how much these additions would affect the amounts consumed or the variation from week to week, but it is likely that the variations would be at least of the same order. With each element, therefore, it is quite possible for one week's record of dietary intake to give a value 50 % more than another week's. Once again this may well mean that a person is judged as having an inadequate supply of an essential nutrient when, over a longer period, the supply is in fact adequate.

Vitamins. Least variation is found with vitamins of the B group, but it is sufficient to lead to error in assessing dietary adequacies. Vitamin C varies more, and a week's intake is certainly not sufficient to give an indication of the usual intake. This is quite apart from the fact that, in Britain, there is an appreciable seasonal variation as the availability of vegetables and fruits changes.

The greatest variation was found with vitamins A and D. In both instances, this was due to the consumption, at relatively long and irregular intervals, of exceptionally potent sources of one or the other of these vitamins.

The results for vitamin A make it necessary to reconsider the conclusions drawn in an earlier study (Doraiswami & Yudkin, 1948). This reported the relationship between dietary vitamin A and dark adaptation. Dietary intakes were measured as in the present investigation, by postgraduate students weighing their food for 1 week and calculating nutrients from food tables. It was found that, out of eleven students investigated, only those three whose average daily intake was below 2500 i.u. had a 'vitamin A-labile' dark adaptation. Since it has now been shown that a week's study may give quite unrepresentative values for the intake of vitamin A, this pleasing correlation between dietary vitamin A and deficiency—as shown by impaired dark adaptation—must be considered fortuitous.

Relation of intake to recommended allowances

Calories. The position of calories in relation to recommended allowances is different from that of the nutrients. With the latter, an amount above the minimal requirement is recommended so as to provide a margin of safety; excess is either stored, metabolized or excreted. The intake of calories should exactly match requirement, since excess must lead to increase in weight. Suggested allowances for calories, therefore, even

more than those for nutrients, are to be considered as average and not as absolute values for every individual.

Some guide as to whether a person's intake of calories matches his requirements is provided by the basal metabolic rate. With persons of similar activity, such as those in this investigation, one would expect that the calories provided by the diets would be to about the same extent above those needed for basal metabolism. The basal metabolism for the six subjects of this study has been calculated from their height and weight (Table 7). Results show that, in four of the subjects, the average intake of

Table 7. *Comparison between calculated 24 hr. basal metabolism and average daily caloric intake of the six subjects*

Subject no.	Calculated basal metabolism (Cal.)	Average daily intake (Cal.)	Difference	
			Cal.	As percentage of basal metabolism
1	1430	2410	980	69
2	1445	2165	720	50
3	1620	2450	830	51
4	1620	2060	440	27
5	1260	1390	130	7
6	1640	2470	830	51

calories was greater than the calculated basal metabolism by 50 % or more. Although the calculated basal metabolism may have been different from that obtained by direct estimation, the smaller difference in subject no. 4, and the much smaller difference in subject no. 5, suggest strongly that even the 4 weeks' average intake was, for these two subjects, probably lower than their usual intake. It is especially difficult to imagine how subject no. 5 could continue for long with an intake of energy only a trifle more than she needed for basal metabolism. It is unfortunate that the subjects were not weighed at the beginning and at the end of the study, for one would have expected subjects nos. 4 and 5 to have lost weight during the survey. The only information available is that subject no. 5, an Indian girl, lost about 10 lb. in weight during the first 6 months of her stay in England. This period began in September 1948 and so included the period of the survey. It might again be mentioned, however, that both subjects nos. 4 and 5 were subjectively well during the period of study.

Nutrients. Although for reasons discussed earlier we cannot accept with complete confidence the absolute figures of intake of nutrients, it is nevertheless worth briefly commenting on the relation between these and the recommended allowances.

Let us assume that the average intake over the 4 weeks is reasonably representative of the usual intake of the subjects (with reservations, as indicated, for subjects nos. 4 and 5). The results show that, apart from subject no. 5 and in one instance subject no. 6, all the subjects had an intake of protein, iron, vitamins A, B₁ and C, and nicotinic acid approaching or exceeding the B.M.A. standards. The intakes of calcium and riboflavin, however, were below the B.M.A. standards. As regards calcium, the discrepancy was small and was no doubt made up by calcium from water. Since, for many nutrients, the N.R.C. standards are higher than the B.M.A. standards—some-

times much higher—most of the subjects were moderately or considerably below the N.R.C. standards for calcium, vitamins B₁ and C, riboflavin and nicotinic acid. As the subjects were getting many of their meals at the College, they obtained their full rations in addition. They also had more than the usual knowledge of nutrition and food values, so that it is likely that their diets compared favourably with those of the general population. The fact that, in spite of this, the intakes of most or all of them fell so far short of the N.R.C. recommendations for several nutrients lends support to the opinion already commonly held in this country that these recommendations are unnecessarily high.

We conclude, therefore, that the problem of relating intake to requirement is much more difficult than is usually admitted. We have first the fact that it is not possible to know with any exactitude the requirements for the nutrients of any one person (Yudkin, 1948). It is now also clear that the accurate assessment of a person's representative dietary intake not only involves laborious techniques if serious error is to be avoided, but also a prolonged period of study because of variations in food consumption.

SUMMARY

1. The diets consumed by six young women have been studied for 4 consecutive weeks and, in two instances, for a further week after a short interval. The diets were weighed by each subject and the calories and nutrients in them calculated from food tables.
2. The weekly intake of calories and nutrients showed considerable variation. The extent of the variation differed with the different dietary components and with the different subjects. For example, in the week of highest intake, one subject took 2 % more calories than in the week of lowest intake, whereas another took 68 % more calories. With vitamin A, the values in the highest week were from two to five times as much as those in the lowest week.
3. It is thus possible for a person to have an intake of any of the dietary components which is apparently adequate in one week and inadequate in another.
4. It is concluded that a dietary survey extending over 7 days cannot be considered to give a sufficiently accurate assessment of the average intake of calories or nutrients by an individual.

I am indebted to Messrs Allen and Hanburys Ltd., The British Drug Houses Ltd., and Messrs H. W. Carter and Co. for grants towards the expenses of this work. My thanks are also due to the subjects of this study for their co-operation, and to Mrs B. Winstanley for aid in the dietary calculations.

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The Evaluation of Leanness-Fatness in Man: Norms and Interrelationships

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One of the fundamental characteristics of man's state of nutrition is the composition of his body. Grossly, at least, this means the proportion of his body-weight accounted for by the basic components of blood (subdivided into plasma and cells), interstitial fluid, bone, fat, and 'active tissues' (principally muscles, glands, and nerves). Body fat shows the most striking variations in states of nutrition varying along the emaciation-obesity continuum. The provision of improved methods for a quantitative estimation of the relative fatness is one of the pressing tasks of nutritional science; it is essential both for the evaluation of calorie nutrition and for the establishment of valid estimates of calorie requirements (cf. Food and Agriculture Organization of the United Nations, 1950).

In the past, the evaluation of fatness has been based almost exclusively on the individual's deviation from some 'standard' reference weight for his sex, age and height. This conforms to popular ideas; a 'fat' person is thought of as a heavy, overweight individual. Clinical monographs on obesity (Rony, 1940; Ryneerson & Gastineau, 1949) devote little or no space to a quantitative evaluation of the degree of obesity. This is defensible if one is concerned only with very marked deviations from normal where, in the absence of oedema, a large increase in weight is likely to mean simply the accumulation of body fat. Experience with animals generally supports the concept of overweight as 'fatness'. A very heavy pig or goose will not only have a larger total weight than a lean animal of the same breed but, at the same time, a disproportionately large percentage of their body-weight will be accounted for in terms of deposited fat.

In man, variations in physical activity may alter markedly the composition of the body and disturb the relationship between the relative body-weight and fatness so that the relative body-weight (obtained by expressing the actual weight as a percentage

of 'standard' weight) for height, sex and age may be grossly misleading as an index of fatness. Welham & Behnke (1942), in their study of professional football players, demonstrated that 'overweight' cannot be simply identified with 'obesity'. The football players were actually 'thin', in the sense of having a low fat content of the body, although the average body-weight was 24.6 % above the army standard for men of the same height and age.

On the lower ('lean') end of the scale, the relationship between relative body-weight (underweight) and leanness may be more stable. In all field reports from areas where there was undernutrition and semi-starvation, including observations made during and after the second world war, marked decrements in body fat have been noted. Unfortunately, almost all of these reports present only qualitative descriptions of the semi-starvation changes. If quantitative data are presented, they are limited to body-weight.

In the Minnesota starvation-rehabilitation experiment (Keys, Brožek, Henschel, Mickelsen & Taylor, 1950) the weight decreased within 6 months from 69.39 to 52.57 kg. (a decrement of 24.2 % of the original value), while the body fat, estimated on the basis of specific gravity, decreased from 9.84 to 3.05 kg. (a change of 69.0 %) (Brožek, 1946). In the rehabilitation period the recovery of the body fat was more rapid than the recovery of the other soft tissues. In the caloric groups, maintained on supplements differing by successive steps of about 400 Cal., the total body-weight was regained to the extent of 21, 30, 41 and 56 % of the semi-starvation loss, whereas for body fat the recovery of 17.0, 42.5, 45.9 and 94.8 % was achieved in this same period.

The method of estimating body fat from specific gravity of the body offers an important advance in the quantitative macroscopic morphology of the living man, even though much yet remains to be done in the way of further validation, refinement, and simplification of the technique. Measurements of the skinfolds, which vary in thickness roughly in proportion to the subcutaneous adipose tissue, are being used with increasing frequency as criteria of fatness, but there are as yet no adequate standardized procedures and instruments. Without such standardization the establishment of usable norms for well-defined sectors of the population will be impossible.

A general review of methods for the evaluation of fatness-leanness (obesity-emaciation) of an individual has been published elsewhere (Brožek & Keys, 1950*a*). The present communication offers specific data obtained for two age groups, younger men (college age) and older men (45-55 years). The study is focused on the problem of the norms and the interrelationships between different measures of fatness.

METHODS

Criteria of leanness-fatness

The characterization of man's 'leanness-fatness' in this paper is based on the measurement of (1) specific gravity of the body, (2) thickness of skinfolds, and (3) external dimensions and body-weight. In living man the percentage of the body represented by fat, estimated on the basis of specific gravity of the body, appears to be the best single criterion for characterizing the individual's leanness-fatness. The

skinfolts may be regarded as the next best index. 'Impure' characteristics, such as the abdominal circumference and the gross body-weight, provide still more indirect indicators of the leanness-fatness of an individual.

Specific gravity of the body. The possibility of estimating the amount of body fat from the specific gravity of the body was demonstrated by Behnke and his collaborators (Behnke, 1941-2; Behnke, Feen & Welham, 1942). This technique takes into account both the subcutaneous fat and the deeper fat deposits. This is of importance because the tela subcutanea accounts for much, but not all, of the adipose tissue. A table for converting the values of specific gravity of man to fat content was provided by Rathbun & Pace (1945).

In the present study the body volume, used in the calculation of body density, was obtained on the basis of the Archimedean principle: volume (l.) = weight in air (kg.) - weight in water (kg.). The subjects were seated in a stainless steel support and lowered into a tank of water ($36 \pm 0.5^\circ$). They exhaled maximally through a copper tube and held their breath for 3-5 sec. needed for reading the weight to the nearest 100 g. The procedure and equipment have been described elsewhere (Brožek, Henschel & Keys, 1949).

The value of the under-water weight has to be corrected for the air remaining in the lungs and respiratory passages at the end of maximal expiration. On the basis of the available data the correction factor of 1.5 l. appears as an acceptable approximation of the average value of residual air volume for young men; in individual cases the correction involves an error which rarely exceeds ± 600 ml. The volume of residual air increases, on the average, with age. For the older men, the value of 2.2 l. was used (Brožek, Carlson & Keys, 1951).

Thickness of skinfolts. The skinfolts were picked up between the thumb and the index finger of the left hand and lifted up, taking care that no underlying muscle tissue was included. The 'bite' covered about 8 cm. of the skin. In very obese individuals the distance had to be increased. The thickness, which represents twice the value of the thickness of the skin plus the subcutaneous tissues, was determined by a pair of calipers. The calipers were placed about 1 cm. above the fingers, holding the skinfold lightly and allowing the pressure of the calipers alone to be applied to the skinfold.

It is obvious that the values are dependent, in part, on the characteristics of the instrument used. The area of the contact points of the calipers was about 3 sq.mm. and the initial (opening) tension was 110 g. (35.4 g./sq.mm.). There was only a small increase in the pressure when the jaws of the calipers were opened more widely. Over the range of skinfold thicknesses encountered in this study the effective tension increased linearly (7.8 g./cm. jaw opening). This variation did not affect appreciably the skinfold values as shown by studies with special calipers on a group of twelve young men. The skinfolts were measured at five points: (1) abdomen, to the right of the navel, (2) chest, above and to the right of the right nipple, (3) back, below the right scapula, (4) arm, on the back, half-way down the upper arm, (5) thigh, above the knee cap.

Relative body-weight. In evaluating the degree of 'overweight', the subject's actual

body-weight was expressed as a percentage of the standard reference weight for the man's age and height. The ages were calculated in terms of the nearest birthday. The weight was measured to the nearest 100 g. The weights in Table IV of the Medico-actuarial Mortality Investigation, abbreviated as M.A.I. (Association of Life Insurance Medical Directors and the Actuarial Society of America, 1912, p. 38) served as standards of reference. This table has been reprinted by Davenport (1923) and cited in numerous other publications, frequently without reference to the original source (see, for example, McLester, 1943, p. 771).

The M.A.I. data have no magic properties and are by no means to be considered as 'ideal' weights, a quality which can be established only on the basis of thorough morbidity and mortality studies, but for 40 years they seem to have been reasonably good average values for 'normal', i.e. non-diseased, Americans. The figures derived from a sample of 100,000 white registrants for the draft, measured in 1940 and 1941, with an average age of 26 years (Edwards, McGill & Rowntree, 1943) agreed closely with the M.A.I. reference weights for men 26 years old.

Body dimensions. The chest and the abdominal circumference were measured with a steel tape at the end of normal expiration. For some purposes the absolute values of these dimensions or their ratios to height are of interest. In the present study the difference between the thoracic and abdominal circumference was used. Behnke *et al.* (1942) and Sarkisian (1946) pointed out that this difference tends to parallel the specific gravity and can serve as a gross measure of fatness-leanness.

Subjects and conditions

In selecting the subjects several criteria were applied: (1) health, the subjects were to be free of discoverable disease; (2) sex, only men were included; (3) age, the sample consisted of two age groups: younger adults (college age) and older adults (ages 45-55 years); (4) occupation, the younger men were students at the University of Minnesota, the older men were business and professional men in Minneapolis and St Paul; (5) relative weight, a fairly wide range was desired.

For the purposes of examining the relationship between fatness and the cardiovascular functions a larger number of overweight individuals was included in the sample than would be likely to be found in a random sample of the population. In the student group this resulted in a distribution of relative body-weights that was slightly skewed toward the higher values. The mean weight of the original group of 159 normal students was 73.3 kg., or 106 % of the 'standard' weight (69.1 kg.) for age (20.4 years) and height (177.8 cm.).

In selecting the older group for the cardiovascular study, effort was made to obtain a rectangular distribution, with approximately equal numbers of subjects in the five categories according to the relative body-weight (group A = below 85.0 % of the standard age-height-weight; B = 85.0-94.9; C = 95.0-104.9; D = 105.0-114.9; E = 115.0 % and above). To this sample was added a group of men certified as 'physically active' by the directors of local athletic clubs. For the 223 physically normal older men (average age 49.2 years) the actual mean weight (74.9 kg.) was close

to the 'standard' weight (75.9 kg.) for height (175.9 cm.), but the distribution was not representative of any definable population.

For the purpose of arriving at norms of fatness-leanness we have selected from each of our two groups a subsample which followed closely, with reference to the relative body-weight, the distribution of this characteristic in a random sample of a well-defined population. Through the courtesy of Dr Ruth Boynton, director of the Student Health Service, University of Minnesota, we were able to obtain the distribution of relative weights in two random samples of students (age 18–26 years) registering at the

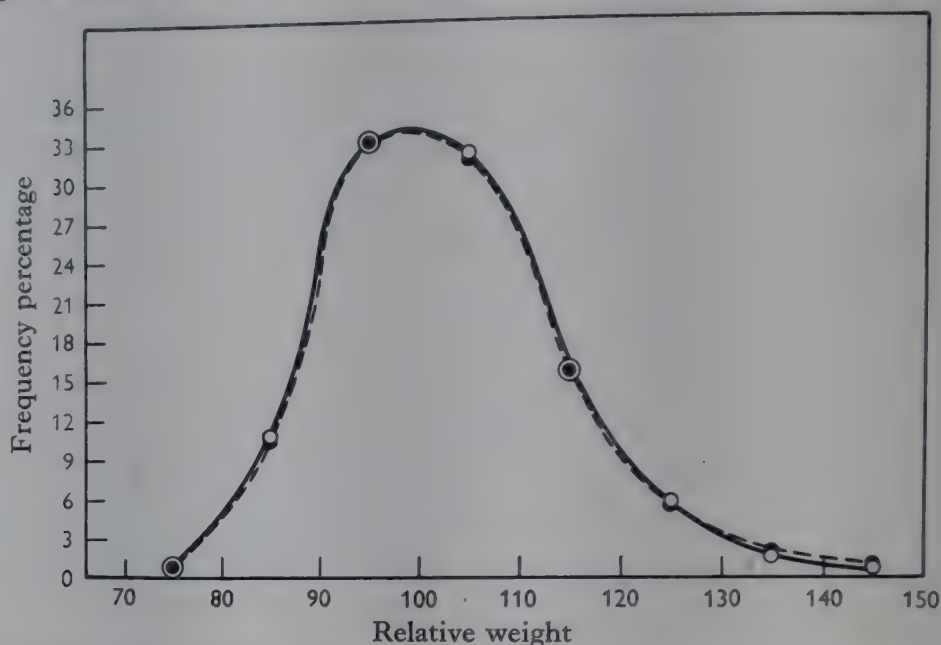


Fig. 1. Distribution of the relative weights in a random sample of University of Minnesota students ($N=7116$, solid circles) and in a matched sample ($N=133$, open circles) used for establishing the norms of the fatness criteria.

University. The distributions obtained for 2 successive years were almost identical. We have used the combined sample ($N=7116$) and selected out of our population of young subjects a subsample that matched the distribution of relative weights in the student population. Within the intervals of relative weight the subjects were selected at random.

For the older men no data on the distribution of relative weight in a random sample of business and professional men, free of disease, could be found in the literature. In order to approximate a random sample we have combined the data for all of the 566 business and professional men with whom we have had contact and who were judged to be free from disease. In making up the subsample for the derivation of norms of fatness for this age group the subjects were selected so as to match the distribution of relative weights found in this sample of 566 men.

The model and the matched distributions for the younger and older subjects are given in Figs. 1 and 2.

Norms

RESULTS

The norms were derived from the frequency distributions of the single criteria of fatness. When the distributions are 'normal', in the statistical sense, the norms can be based effectively on the mean and the standard deviation of the values in the sample. When this is not the case the norms may be based on percentiles.

For the purposes of classification of the subjects into categories of fatness, we have used the twentieth, fortieth, sixtieth and eightieth percentile. These limit values are sometimes called 'quintiles'. They divide the sample into five equal groups, as far as the number of individuals in each fatness category is concerned. The quintiles, together with the fiftieth percentile (the median) are given in Tables 1 and 2. For completeness, the means and standard deviations are also indicated. It may be noted that some of the distributions of fatness measures for the younger men showed considerable skewness.

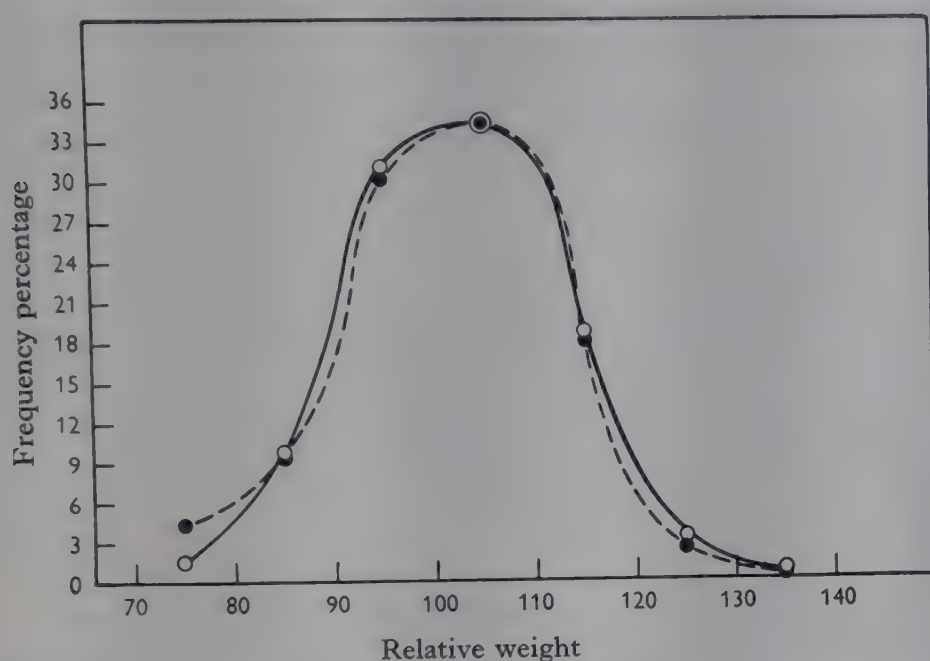


Fig. 2. Distribution of the relative weights in a group of older men (45-55 years of age, $N=566$, solid circles) who volunteered as subjects for the cardiovascular study and in a matched sample ($N=122$, open circles) used for establishing the fatness criteria.

Table 1. Normative data on indices of leanness-fatness for male college students

($N=133$; mean age = 20.3 years, S.D. = 1.9)

Criterion	Percentiles				Median	Mean	S.D.
	20	40	60	80			
Relative body-weight	91.6	97.0	102.7	113.6	99.7	101.41	11.94
Specific gravity	1.0893	1.0820	1.0746	1.0645	1.0781	1.0766	0.0141
Percentage of body fat	4.92	8.40	11.90	16.77	10.25	10.93	—
Skinfolds (mm.):							
Abdomen	11.1	14.5	19.1	24.8	16.2	18.2	8.4
Chest	9.5	12.3	16.1	22.5	14.2	15.9	7.4
Back	9.4	11.8	14.8	19.0	13.3	14.3	5.8
Arm	6.6	9.1	11.6	14.6	10.2	10.9	4.6
Thigh	5.6	7.6	9.1	11.0	8.5	8.6	3.7
Chest circumference — abdominal circumference (cm.)	17.1	15.0	12.2	9.4	13.4	13.2	5.1

We have available three other distributions of specific gravity with incomplete information on the distribution of relative weight and age. These data were obtained for ninety-nine Navy men between 20 and 40 years of age (Behnke *et al.* 1942), for a similar sample of seventy-five Navy men (Welham & Behnke, 1942), and for thirty-two

Table 2. Normative data on indices of leanness-fatness for middle-aged men

(N=122; mean age=49.0 years, S.D.=2.8)

Criterion	Percentiles				Median	Mean	S.D.
	20	40	60	80	50		
Relative body-weight	92.4	98.7	104.6	111.6	101.5	101.8	11.31
Specific gravity	1.0656	1.0590	1.0523	1.0460	1.0560	1.0554	0.01182
Percentage of body fat	16.3	19.5	22.7	26.0	21.0	21.3	—
Skinfolds (mm.):							
Abdomen	18.6	23.1	26.9	31.5	25.0	25.5	7.8
Chest	18.4	23.1	26.4	31.3	24.7	24.5	7.8
Back	14.2	17.7	20.8	24.5	19.3	19.9	7.1
Arm	10.9	13.4	14.9	18.2	14.1	14.4	4.2
Thigh	8.0	9.6	10.9	12.7	10.1	10.3	3.0
Chest circumference — abdominal circumference (cm.)	11.4	8.0	5.7	4.1	6.8	7.2	4.6

Table 3. Distribution of specific gravity in 206 healthy young men, 20-40 years of age

Specific gravity	Navy men (99)	Navy men (75)	Conscientious objectors (32)	Total f	f %	Σf %
1.020-1.029	2	—	—	2	0.97	100.00
1.030-1.039	2	1	—	3	1.46	99.03
1.040-1.049	4	1	1	6	2.91	97.57
1.050-1.059	20	11	5	36	17.48	94.66
1.060-1.069	23	18	7	48	23.30	77.18
1.070-1.079	27	14	12	53	25.73	53.88
1.080-1.089	14	16	7	37	17.96	28.15
1.090-1.099	7	14	—	21	10.19	10.19

conscientious objectors (Keys *et al.* 1950), 20-33 years of age, mean age 25.5. The data are summarized in Table 3.

The mean is 1.0711 (corresponding to 13.6 % of the body as fat), the median 1.0714, the standard deviation 0.0145. The twentieth, fortieth, sixtieth and eightieth percentiles are 1.0845, 1.0753, 1.0653 and 1.0583. These figures fall between those for the college and the business and professional groups. They were obtained on healthy young men and may perhaps be regarded as an approximation to 'ideal' values of fatness in the adult man. An increase in fatness with age appears undesirable even though, statistically, a continuing rise in the relative amount of body fat is a 'normal' phenomenon.

Estimation of the total fatness-leanness from single variables

One of the aims of this study was to develop equations for estimating the total fatness from more easily accessible measures of fatness than the specific gravity. The prediction equations are based on the means, standard deviations and the coefficients of correlation, but for application to calculations we have converted these to the simple form, $\hat{Y}=a+bX$. These prediction equations for the several indirect fatness criteria are presented in Table 4.

Table 4. *Equations for prediction of specific gravity of the body ($\hat{Y}=a+bX$) on the basis of single fatness criteria*

(The skinfold values are in mm.)

Variable	Group	
	Younger men	Older men
Skinfolds:		
Abdomen	$1.0996-0.001398X_1$	$1.0783-0.000851X_1$
Chest	$1.0984-0.001586X_2$	$1.0810-0.001039X_2$
Back	$1.1012-0.001770X_3$	$1.0791-0.001148X_3$
Upper arm	$1.1034-0.002313X_4$	$1.0824-0.001840X_4$
Thigh	$1.0155-0.003209X_5$	$1.0789-0.002172X_5$
Relative body-weight	$1.1588-0.000787X_6$	$1.1168-0.000605X_6$

The coefficients of correlation between specific gravity and other criteria of fatness-leanness are presented in Table 5. The effectiveness of estimating Y from X does not increase linearly with increasing values of r . For this reason it is preferable in interpreting the meaning of a correlation coefficient to express the ‘goodness of prediction’ in terms of the index of forecasting efficiency ($F.E.$)*. This index gives directly the percentage of reduction in the error of prediction for a given r . These data are also given in Table 5.

Table 5. *Coefficients of correlation (r) between specific gravity and other criteria of fatness-leanness for younger ($N=116$) and older ($N=214$) men, indices of forecasting efficiency ($F.E.$), and standard errors of estimate of the specific gravity ($S.E.E.$)*

Variable	Group					
	Younger men			Older men		
	r	$F.E.$	$S.E.E.$	r	$F.E.$	$S.E.E.$
Skinfolds:						
Abdomen	-0.839	45.6	0.00800	-0.596	19.7	0.01027
Chest	-0.857	48.5	0.00757	-0.682	26.9	0.00935
Back	-0.809	41.2	0.00864	-0.681	26.8	0.00936
Upper arm	-0.828	43.9	0.00825	-0.647	23.8	0.00975
Thigh	-0.749	33.7	0.00975	-0.538	15.7	0.01078
Relative body-weight	-0.783	37.8	0.00914	-0.633	22.5	0.00991

The coefficients of correlation are lowered (attenuated) by the ‘errors of measurement’. In addition to the effect of variation in the readings present in all types of measurement, the values of specific gravity are subject to the error arising from the fact that the volumes of residual air were not determined for every individual, but an average value was used.

* In a simplified form $F.E.=100 [1-\sqrt{(1-r^2)}]$. It is based on the standard error of estimate, $S.E.E.=S.D._Y\sqrt{(1-r^2)}$, expressed as percentage of the standard deviation of the Y values ($S.D._Y$):

$$F.E.=100-100\frac{S.D._Y\sqrt{(1-r^2)}}{S.D._Y}.$$

Total fatness-leanness estimated from combined variables

The accuracy of prediction may be raised by combining several prediction variables. The simplest, but not the most effective, approach is to consider all estimates as having the same validity (i.e. assign to each of them a 'weight' of 1) and average the several estimates obtained. The technique of simple averaging does not allow a precise evaluation of the reduction in the error of estimate resulting from pooling several prediction variables. This may be achieved by predicting the specific gravity on the basis of a multiple regression equation. This technique takes into account both the correlations of each variable with the criterion (i.e. the specific gravity) and the intercorrelations between the variables. In combining the measurements, economy of effort calls for variables that have a high correlation with the criterion but low correlation with the other prediction variables. The basic correlation coefficients are presented in Tables 6 and 7.

Table 6. *Intercorrelations between indices of fatness for younger men*

(N=116; mean age=21.9 years, S.D.=2.0)*

	(Y)	(X ₁)	(X ₂)	(X ₃)	(X ₄)	(X ₅)	(X ₆)
Specific gravity	—	-0.839	-0.857	-0.809	-0.828	-0.749	-0.783
Skinfolds:							
Abdomen	-0.839	—	+0.938	+0.900	+0.853	+0.752	+0.804
Chest	-0.857	+0.938	—	+0.914	+0.858	+0.766	+0.832
Back	-0.809	+0.900	+0.914	—	+0.827	+0.768	+0.864
Arm	-0.828	+0.853	+0.858	+0.827	—	+0.803	+0.780
Thigh	-0.749	+0.752	+0.766	+0.768	+0.803	—	+0.776
Relative body-weight	-0.783	+0.804	+0.832	+0.864	+0.780	+0.776	—

* The correlations were computed from measurements obtained during the 2nd year of the study.

Table 7. *Intercorrelations between indices of fatness for older men*

(N=214; mean age=49.2 years, S.D.=2.8)*

	(Y)	(X ₁)	(X ₂)	(X ₃)	(X ₄)	(X ₅)	(X ₆)
Specific gravity	—	-0.596	-0.682	-0.681	-0.647	-0.538	-0.633
Skinfolds:							
Abdomen	-0.596	—	+0.794	+0.758	+0.633	+0.550	+0.693
Chest	-0.682	+0.794	—	+0.799	+0.717	+0.542	+0.692
Back	-0.681	+0.758	+0.799	—	+0.730	+0.602	+0.752
Arm	-0.647	+0.633	+0.717	+0.730	—	+0.692	+0.644
Thigh	-0.538	+0.550	+0.542	+0.602	+0.692	—	+0.634
Relative body-weight	-0.633	+0.693	+0.692	+0.752	+0.644	+0.634	—

* The correlations were computed from measurements made on all older men for whom specific gravity values were available and who were clinically normal.

The values needed for the multiple prediction equation were obtained by the Doolittle method (Johnson, 1949, p. 327). The general formula of the equation is

$$\hat{Y} = a + b_1 X_1 + b_2 X_2 + \dots + b_n X_n,$$

where the *b* values are 'weights' yielding the best estimate of the predicted variable, $\hat{Y}$, obtained by a linearly additive combination of the prediction variables. Technically,

the b values are referred to as partial regression coefficients. The value a is a constant, defined as

$$a = \bar{Y} - (b_1 \bar{X}_1 + b_2 \bar{X}_2 + \dots + b_n \bar{X}_n).$$

Using six variables, we obtain the following equations for predicting the specific gravity of the younger and older men, respectively (equations 1 and 2):

$$\begin{aligned} \hat{Y} = & 1.1125 - 0.000292X_1 - 0.000661X_2 + 0.000181X_3 \\ & - 0.000711X_4 - 0.000375X_5 - 0.000122X_6, \end{aligned} \quad (1)$$

$$\begin{aligned} \hat{Y} = & 1.0967 + 0.000042X_1 - 0.000423X_2 - 0.000320X_3 \\ & - 0.000511X_4 - 0.000247X_5 - 0.000156X_6. \end{aligned} \quad (2)$$

The coefficients of multiple correlation between specific gravity and the six prediction variables are $R_{Y \cdot 123456} = 0.8760$ for younger, and $R_{Y \cdot 123456} = 0.7441$ for the older men. This corresponds to the standard errors of the specific gravity estimates of 0.00708 and 0.00854, and indices of prediction efficiency of 51.8 and 33.2 %.

In order to reduce the labour involved in making the actual measurements and in predicting the specific gravity, those variables that did not contribute significantly to the accuracy of prediction were removed. Johnson (1949, p. 339) presents in detail the steps involved in testing the statistical significance of the partial regression coefficients and in calculating the simplified prediction equations. On this basis, the following prediction equations were derived for the younger and older men (equations 3 and 4), respectively:

$$\hat{Y} = 1.1017 - 0.000282X_1 - 0.000736X_2 - 0.000883X_4, \quad (3)$$

$$\hat{Y} = 1.0967 - 0.000393X_2 - 0.000315X_3 - 0.000598X_4 - 0.000170X_6. \quad (4)$$

The accuracy of predicting specific gravity of the younger men from equation (3) is characterized by $R = 0.8709$, standard error of estimate = 0.00722, and $F.E. = 50.9$ %. For the older men (equation 4), $R = 0.7430$, standard error of estimate = 0.00856, and $F.E. = 33.1$ %.

DISCUSSION

Implications of the data for nutritional research

Quantitative morphology of the body mass, separated into its primary components, provides the initial framework for the description of man's nutritional status. In living man, partitioning of the body into the principal tissues, including body fat, must be carried out largely by indirect methods.

For classifying individuals several criteria may be used: body-weight related to the 'standard' weight for age, sex and height; external body dimensions, either singly or, preferably, in combinations such as the difference between the circumference of the chest and the abdomen; skinfolds; specific gravity. Norms were provided here for classification of normal men of college age and of business and professional men in the age bracket of 45-55 years, using eight criteria of fatness. These data should provide useful reference material for other investigators. Extension of the work to other age levels and to women is much needed.

The validities of these criteria differ. Final validity can be established directly only on the basis of correlating the values of the particular criterion with subsequent analyses of the actual fat content of the body. Technically, any attempt at such a validation would meet almost insuperable obstacles. Quantitative analyses of cadavers are conspicuous by their rarity (see Mitchell, Hamilton, Steggerda & Bean, 1945).

In the present study the validation of the more indirect or partial indicators of fatness was carried out in terms of correlations with the specific gravity. The chest skinfolds showed the highest correlation with specific gravity in both age groups ($r = -0.857$ for the younger men, $r = -0.682$ for the older men). The relative weight showed a surprisingly high correlation (-0.783 and -0.633 , respectively). However, it should be noted that this relationship holds only within a limited age range. We have pointed out (Brožek & Keys, 1950*b*) that older men have a higher fat content at the same relative weight. In a group of thirty-seven younger men (mean age 22.1 years) and sixty-six older men (mean age 44.1 years) who were within $\pm 5\%$ of their standard weight (mean relative weight of 100.2 and 100.0) the values for mean body fat, estimated from specific gravity corrected for residual air in the lungs, were 9.8 and 21.0 %.

At times, information on the absolute amount of body fat is desirable. For example, one may wish to refer the basal metabolic rate not to the gross body-weight (or to body surface, estimated from body-weight and height), but to the fat-free body mass. In the present paper, equations were provided for estimating total fat from the less direct criteria of fatness, both singly and combined into a multi-variable prediction equation. The estimation equations were developed for the specific gravity rather than for the total storage fat. In a way it would be easier to think directly in terms of the estimated body fat rather than the specific gravity; for one, the correlations with the majority of the indices of body fat would be positive. However, it may turn out in the course of further research that the conversion tables worked out by Rathbun & Pace (1945) will need revision. Such a revision would not affect the prediction equations developed in this paper.

Skinfolds, selection of points

In the present study the points at which the skinfolds were to be measured were selected on *a priori* grounds. The following conditions were to be satisfied: (1) representation of regions known to show large variations in subcutaneous fat (abdomen, chest), (2) representation of the extremities (arm and thigh measurements) and (3) ease of precise location.

The information provided here throws much-needed light on the value of different skinfolds as predictors of the total body fat. However, a valid empirical selection of the locations of a small number of skinfolds out of an infinite number of possible points still remains to be carried out. In addition to fulfilling such a requirement as accessibility, the points at which the skinfolds are to be measured must have a definite location, facilitating repeatability of the measurements. The selected measurements should correlate highly with the total body fat, while having relatively low correlations with each other.

Intercorrelation between the criteria of fatness-leanness

Batkin (1915) pointed out that the thickness of the subcutaneous layer varies at different parts of the body surface but added that, normally, there is a certain parallelism in the values obtained in different locations. This statement was not documented. Franzen (1929) obtained correlation coefficients varying from 0.81 to 0.85 for skinfolds measured over the biceps and the triceps of children 10–12 years of age. Reynolds (1945) noted, on the basis of roentgenograms for eight areas of the body, a fairly high association between the thickness of the subcutaneous tissues in different areas, but did not give actual correlation data.

In the younger Minnesota men the correlations of the skinfolds with each other, measured at the five points, varied from +0.752 to +0.938. The corresponding intercorrelations for the older men were generally lower, with a range from +0.542 to +0.799.

Interindividual differences

The amount of body fat exhibits larger differences between normal individuals than perhaps any other body constituent. In fifty guinea-pigs examined by Rathbun & Pace (1945) the fat content varied from 1.5 to 35.8 % of the body-weight. The specific gravity of the eviscerated body ranged from 1.096 to 1.021. The range is almost as large as was reported by Moulton (1920) for beef steers maintained in a good state of nutrition until the animals were 11 months old and subsequently fed at different caloric levels. The carcass of the control animal contained 18.5 % fat, whereas a fattened steer had nearly twice as much (32.6 %). In an animal that lost 0.5 lb. daily for 10 months and became emaciated the fat content was only 1.9 %.

In the present study the 'storage' fat, estimated from the specific gravity, varied in the younger men from 0 to 32.7 % of the body-weight, in older men from 1.4 to 34.2 %; the other fatness criteria also exhibited large individual differences. Part of these differences may be explained as 'error' of measurement, including such items as the failure to measure residual air in each individual, but the major variation is undoubtedly simply interindividual difference.

SUMMARY

1. Several criteria of leanness-fatness were used in the characterization of a representative sample of 133 college men (mean age = 20.3 years, S.D. = 1.9) and 122 business and professional men (mean age = 49.0 years, S.D. = 2.8). Clinically, all men were free of disease.
2. Frequency distribution for relative body-weight, specific gravity, thickness of skinfolds, and the difference between the circumference of the chest and the abdomen were analysed in order to obtain norms of relative fatness.
3. Equations for prediction of specific gravity (and the corresponding percentage of body fat) from single and combined criteria of fatness were developed.
4. Implications of the data for nutritional research are considered, and the urgent need for extension of the studies to other groups is pointed out.

The present communication is a part of a long-range study on the effects of ageing, with emphasis on the cardiovascular system. The work is being supported, in part, by funds provided by the U.S. Public Health Service. We wish to express our appreciation to Mr Kenneth F. Tiede for careful statistical computations.

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Factors Affecting the Utilization of Food by Dairy Cows

4. The Action of the Reticulo-Omasal Orifice

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In any part of the digestive tract the time available for the breakdown and absorption of food is determined by the rate at which food residues pass through that piece of the gut. The reticulo-rumen is a major site of digestion in the cow and the digestion of food, particularly the crude fibre fraction, is affected by changes in the rate of passage of the food through the reticulo-rumen, provided there are no compensating changes in the rate of breakdown of the food (Balch, 1950; Balch & Johnson, 1950). Since undigested food can pass from the reticulo-rumen to other parts of the digestive tract only through the small reticulo-omasal orifice, the movements of this orifice are likely to influence the rate of passage of food.

The material passing through the reticulo-omasal orifice must come from the reticulum and possibly from the anterior part of the rumen. It will, therefore, tend to contain 90–95 % water (Balch, 1950), particles of solid food and micro-organisms in suspension and substances in solution.

Several observations suggest that there is a steady flow of digesta through the reticulo-omasal orifice throughout the day. Paloheimo (1939) slaughtered cows at intervals after giving them chromium sesquioxide, and found that the passage of this very heavy substance to the omasum had begun even in those cows killed immediately after administration. Phillipson (1946) suggested that there must be a constant flow of digesta through the reticulo-omasal orifice because the omasum, abomasum and intestines contain digesta at all times. In sheep the flow of digesta from the abomasum was found to be in gushes with usually less than 15 min. between each gush (Phillipson, 1948), which also suggests a continuous flow of digesta from the reticulo-rumen.

The forces that activate the passage of digesta from the reticulo-rumen to the omasum are not fully understood. Wester (1926) and Schalk & Amadon (1928) considered that the movements of the omasum play an important part in drawing semi-liquid digesta through the reticulo-omasal orifice into the omasum, but Phillipson (1946) did not support this contention. Both Wester and Schalk & Amadon described a sudden fall in pressure at the top of the pressure gradient in the omasum, illustrating the fall with kymograph records of varying clarity. Wester also gave a kymograph record of the contraction of the reticulo-omasal orifice.

The present experiments were undertaken in order to study, in greater detail than was given by Wester or Schalk & Amadon, the relationship between pressure changes

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in the reticulo-rumen, reticulo-omasal orifice, omasum and abomasum and to discover whether these relationships were affected by any of the normal activities of the cow. It seemed likely that such a study would aid understanding of the forces activating the passage of digesta from the reticulo-rumen.

METHODS

The experiments were conducted on the two Shorthorn cows with rumen fistulas that had been used in earlier experiments of this series (Balch, 1950; Balch & Johnson, 1950; Balch & Kelly, 1950). Of these, cow W (Winsome) was receiving a daily diet of 9 kg. hay and cow Y (Halora) one of 7.7 kg. hay and 8.2 kg. concentrates. Cow Y was producing about 16 kg. milk daily.

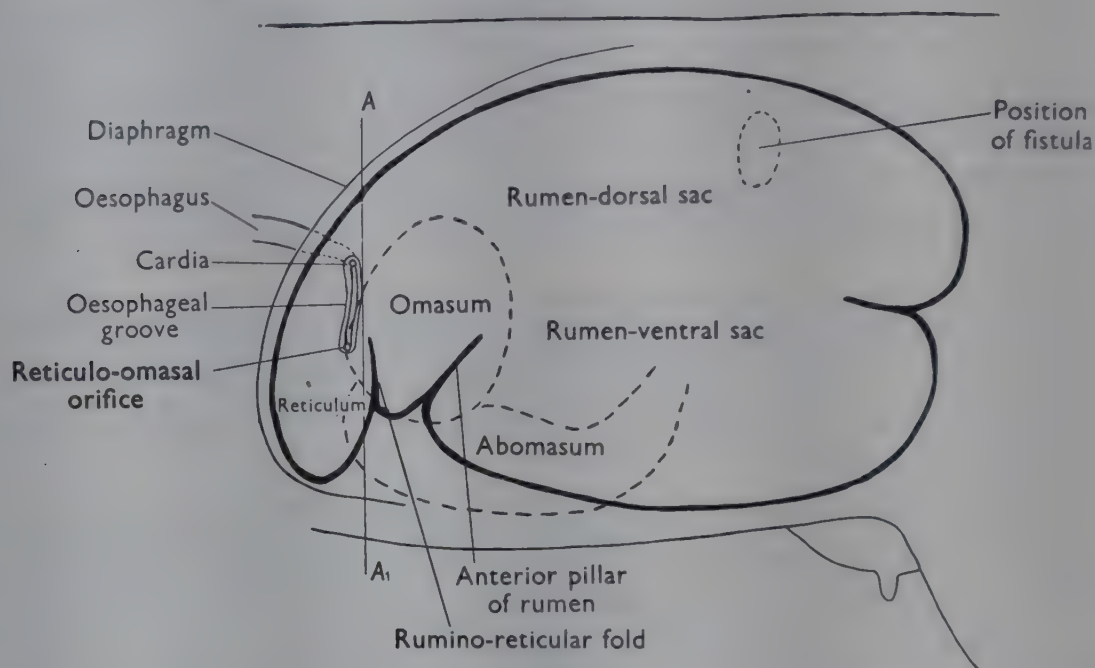


Fig. 1. Diagram showing the approximate position of the reticulo-rumen, against the left side, and the omasum and abomasum, against the right side, in the cow. *A-A*₁ is the approximate position at which the section shown in Fig. 2 cuts the median plane.

Records of gastric movements and of the movements of the reticulo-omasal orifice were made by means of lightly inflated toy balloons operating tambours. The tambours were placed vertically in an Evershed and Vignoles operation recorder (Evershed and Vignoles Ltd., Acton Lane, Chiswick) (Pl. 1) giving ink records on ruled white paper 60 ft. long. With tambours in this position it was necessary to add small weights on short projections at right angles to the levers supporting the writing points. The weights ensured that the levers remained in contact with the triangles of cork on the membranes of the tambours. The entire apparatus, enclosed in a dust-proof cover measuring 50 × 20 × 20 cm., was permanently attached to the cowshed wall.

The position of the reticulo-omasal orifice is shown diagrammatically in Fig. 1. The activity of the orifice was studied in relation to the activity of the reticulum, the anterior pillar of the rumen, the neck of the omasum and the abomasum. The positions of the various balloons are shown diagrammatically in Fig. 2. Balloons were maintained

in the reticulum by means of a 1 kg. brass weight. They were held by hand near the anterior pillar of the rumen and were fixed in the omasum and in the reticulo-omasal orifice by means of an 'anchor'. The 'anchor' consisted of a circle (diameter 5.5 cm.) of 0.3 cm. brass welding rod, which was inserted in the omasum. A 6.5 cm. shank was attached to a cross-piece, forming a diameter of the circle and passed through the orifice. The balloon detecting orifice movements lay along the shank of the anchor. Balloons were placed in the abomasum by means of wire guides but once in position, at a point 60 cm. from the reticulo-omasal orifice, they needed no further support.

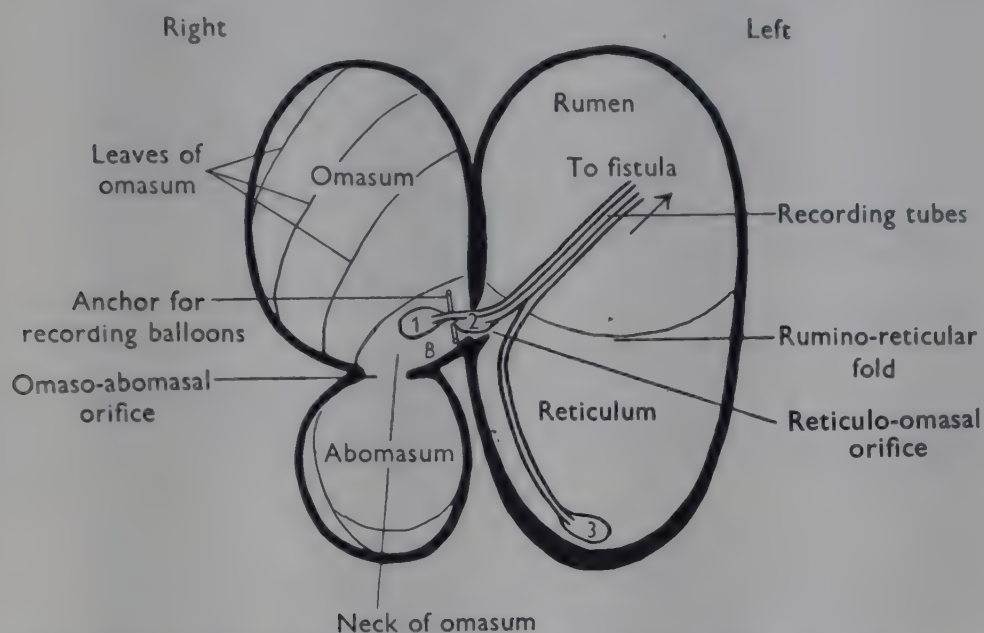


Fig. 2. Diagrammatic vertical cross-section in a slightly oblique plane passing through the reticulo-omasal and omaso-abomasal orifices and cutting the median plane approximately at $A-A_1$ in Fig. 1. The positions of the recording balloons are shown in: 1, the omasum; 2, the reticulo-omasal orifice, and 3, the reticulum. The bridge of the omasum is marked B.

Preliminary estimates of absolute pressures in the reticulum and abomasum were made. For this purpose a single tambour was used, and the recording balloon was replaced by a metal membrane-holder. This holder was shaped like a thistle funnel, a rubber membrane being stretched across the cup (diameter 2.5 cm.), and the recording tube attached to the tube. Before and after recording, the tambour was calibrated by placing the recording surface and tube inside a large vessel, maintained at rumen temperature, in which pressures could be varied. Pressures in this vessel were measured by means of a mercury manometer.

RESULTS

Pressure changes in the reticulo-omasal orifice and in the compartments of the stomach

During rest. Changes of pressure in the reticulum, the reticulo-omasal orifice and the omasum of the two cows while they were standing at rest are shown in Fig. 3. The reticulum showed the usual double contraction at a rate of about sixty/hr. The omasum showed a typical rather slow rise and fall in pressure beginning before the reticulum contractions. Near the peak of high pressure in the omasum there was a sudden and very marked fall which invariably coincided with the second reticular

contraction. This fall was of short duration and the high pressure was regained, usually reaching a somewhat higher peak than had been recorded before the drop. The balloon in the reticulo-omasal orifice recorded a varying amount of movement at the time of the first reticular contraction. At the time of the second reticular contraction there was a drop in pressure in the orifice, immediately followed by a powerful contraction. Palpation showed that the reticulo-omasal orifice was normally loosely open, the first

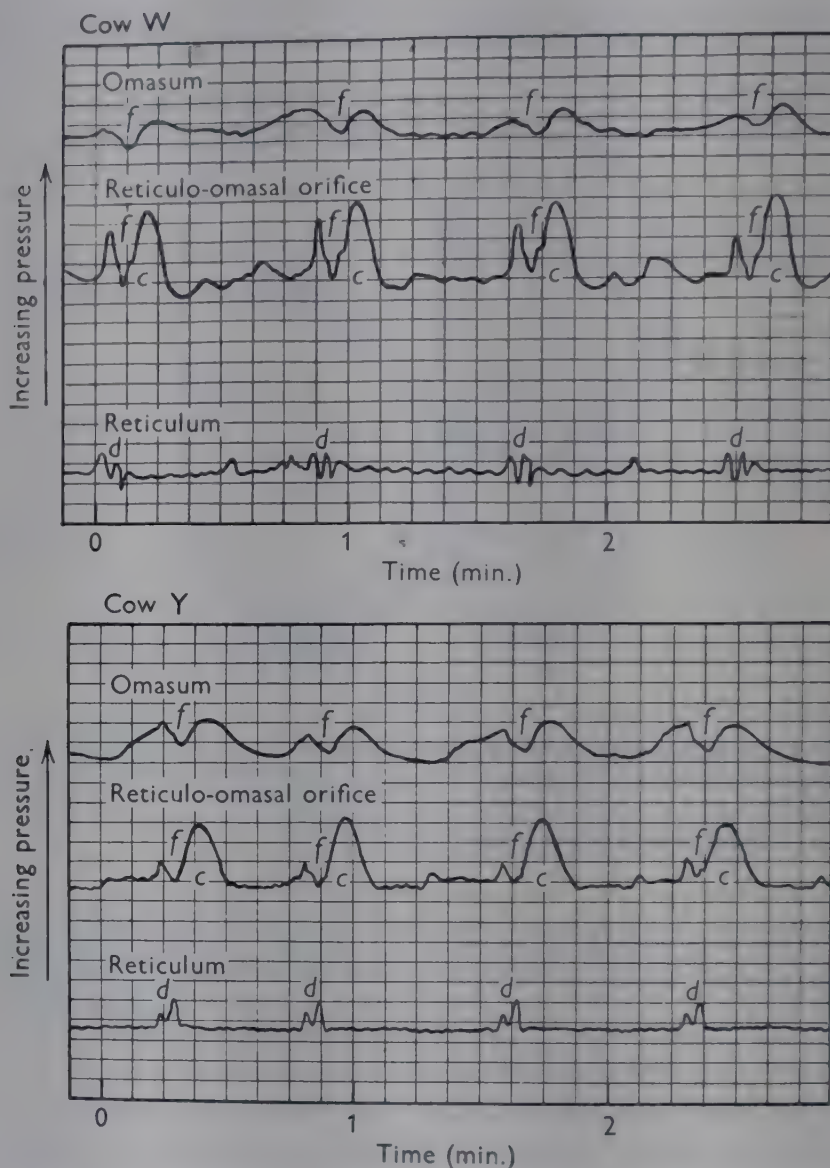


Fig. 3. Pressure changes in the reticulum, the reticulo-omasal orifice and the omasum of two cows at rest. The double contraction of the reticulum (*d*), the sudden fall in pressure in the omasum and in the reticulo-omasal orifice (*f*) and the powerful contraction of the orifice (*c*) are shown.

small peak of pressure recorded by the balloon being due to movement of the surrounding muscular wall. During the first reticular contraction the larger movement was a powerful closing of the orifice. Similar observations were made by Wester (1926).

During eating. During eating the rate of contraction of the reticulum was considerably increased, often reaching a rate of 105 double contractions/hr. However, the movements of the reticulo-omasal orifice and of the reticulum continued to bear the same relationship to the contractions of the reticulum as they did when the cow was at rest.

During rumination. During rumination the reticulum has an extra contraction which precedes the two normal contractions and forms an essential part of the regurgitation reflex. This contraction was recorded by the balloon in the reticulo-omasal orifice as an additional small movement preceding the main contraction of the orifice (Fig. 4). In other respects the relationships of the pressure changes in the reticulum, the omasum

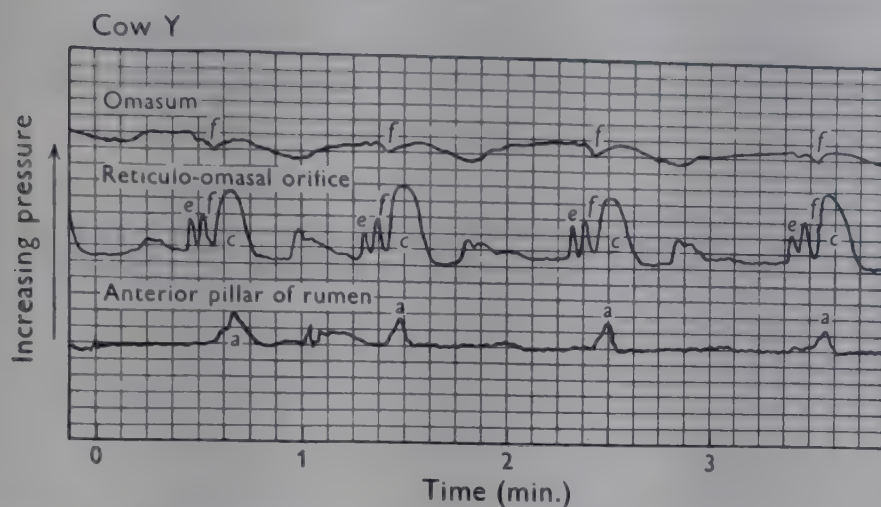


Fig. 4. Pressure changes near the anterior pillar of the rumen, in the reticulo-omasal orifice and in the omasum of cow Y during rumination. The extra contraction of the reticulum during regurgitation (*e*), the sudden fall in pressure in the omasum and in the reticulo-omasal orifice (*f*), the powerful contraction of the orifice (*c*) and the contraction of the anterior pillar of the rumen (*a*) are shown.

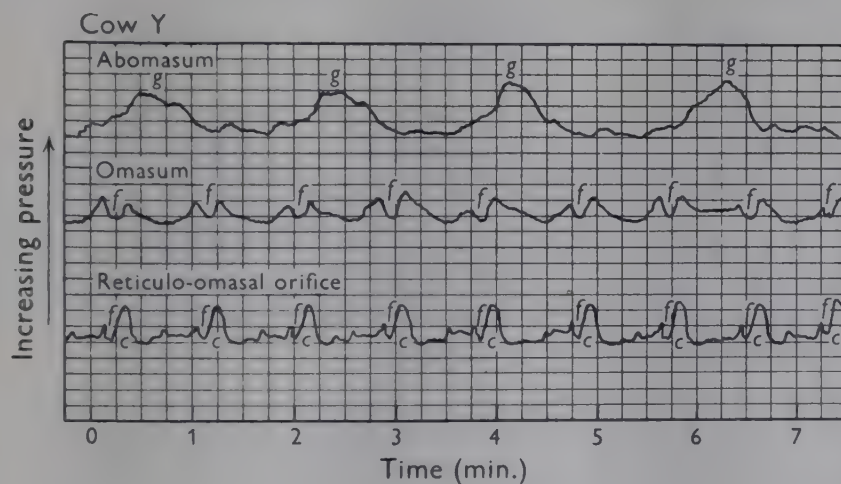


Fig. 5. Pressure changes in the reticulo-omasal orifice, the omasum and the abomasum of cow Y lying at rest. The sudden fall in pressure in the omasum and in the reticulo-omasal orifice (*f*), the powerful contraction of the orifice (*c*) and the peak of pressure in the abomasum (*g*) are shown.

and the reticulo-omasal orifice were the same during rumination and during rest although the cycle of activity occurred typically at about fifty contractions/hr. Fig. 4 shows that the powerful contraction of the reticulo-omasal orifice coincided with the contraction of the anterior pillar of the rumen.

During lying. When the cows lay down there was a slight increase in the basal pressure, and respiratory movements were more prominent in the recordings from all the stomach compartments. The changes of pressure in the various parts continued unchanged in the manner described above (Fig. 5).

During drinking. When the cows drank water, from cowshed drinking bowls, the general pattern of pressure changes in the reticulum, the reticulo-omasal orifice and the omasum remained unchanged (Fig. 6). During drinking the contraction of the orifice appeared to be slightly less powerful than during resting. Frequent palpation established that in these two cows the act of drinking was accompanied by a considerable stiffening and raising of the lips of the oesophageal groove; water flowed down the groove to some extent but could be felt escaping at the lower end into the reticulum and anterior rumen; water did not appear to pass into the omasum to any considerable degree.

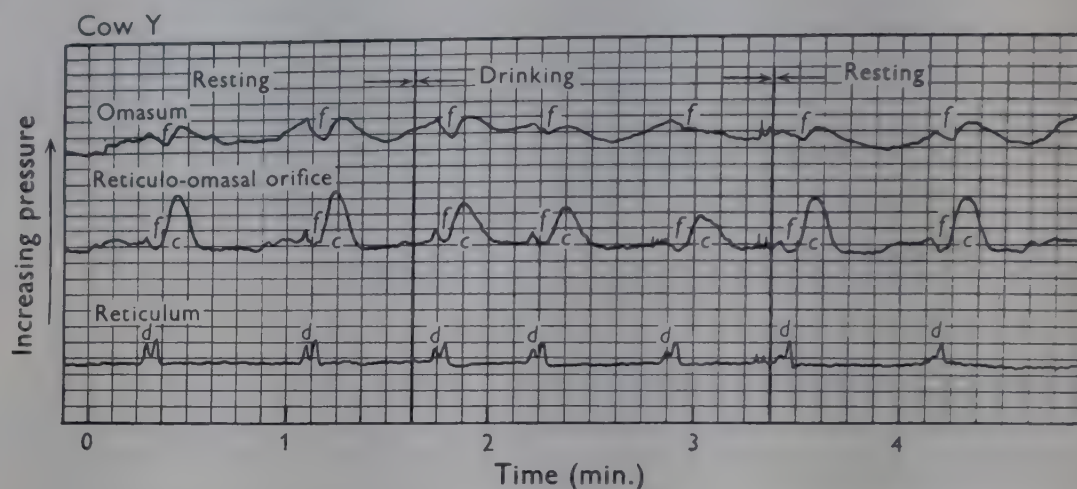


Fig. 6. Pressure changes in the reticulum, the reticulo-omasal orifice and the omasum of cow Y at rest and during drinking. The double contraction of the reticulum (*d*), the sudden fall in pressure in the omasum and in the reticulo-omasal orifice (*f*) and the powerful contraction of the orifice (*c*) are shown.

During milking. During milking there was no characteristic change in the pattern of pressure changes in the reticulum, the reticulo-omasal orifice or the omasum. It was sometimes noticed that when a cow was milked there was a fall in basal pressure in the reticulum and an increase in the amplitude of the double contraction, but similar changes were also observed at other times.

The relationship between the contractions of the reticulo-omasal orifice, of the abomasum and of the anterior pillar of the rumen

The main contraction of the reticulo-omasal orifice coincided with the contraction of the anterior pillar of the rumen (Fig. 4). The data collected in this investigation are insufficient to indicate whether the two movements were linked directly or only indirectly by virtue of their both being initiated by the same pace-maker, which could have been either the reticulum or the omasum.

Only limited recordings of abomasal pressure changes have been made. The abomasum sometimes appeared to have a slow peristaltic type of movement with peaks every 2-4 min. A good example obtained when cow Y was lying down at rest after her evening meal is shown in Fig. 5. These were presumably the peristaltic waves that occur at the pyloric end of the abomasum (McAnally & Phillipson, 1944). There was no apparent relationship between the cycle of movements in the reticulo-omasal orifice and the movements of the abomasum.

Absolute pressures in the reticulum, omasum and abomasum

The recording of absolute pressures in these organs was considerably hampered by shifting of the base-line after calibration, due apparently to expansion of the air in the recording tube caused by the heat of the animal's body. This difficulty was partly overcome by calibrating in a vessel at rumen temperature. The results, which must be regarded as preliminary estimates, suggest that the mean pressure in both the reticulum and abomasum at the time of recording, which was always between the hours of 9.0 a.m. and 6.0 p.m., was within the range 2.5–3.5 cm. mercury above air pressure. During the double contraction of the reticulum, increases in pressure of 1–2 cm. mercury were recorded. The pressure in the abomasum varied from its mean by about ± 0.7 –1.0 cm. mercury. This suggests that for periods of perhaps 2 min. at a time the pressure in the abomasum was lower than the basal pressure in the reticulum. At the time of the reticular contraction, pressure in the abomasum was also usually lower than that in the reticulum.

Movement of small supported balloons and of the recording capsule used for estimating absolute pressures in the omasum, where they had remained for a considerable period, to the reticulum showed that the characteristic drop in pressure at the height of the wave of pressure in the omasum was frequently to a pressure lower than that recorded in the reticulum at the time of the last reticular contraction.

DISCUSSION

The movements of the reticulo-omasal orifice were shown to bear a very constant and characteristic relationship to the pressure changes in the reticulum and omasum. The pattern was found to continue without interruption when the animal was resting, eating, ruminating, drinking, lying down, or being milked.

The reticulum had the usual short, powerful, double contraction, preceded in rumination by an additional contraction at the time of the regurgitation. The omasum exhibited a slow rise and fall in pressure with a highly characteristic drop in pressure at about the highest point. This drop never exceeded the main fall between cycles of activity and coincided with the contraction of the reticulum. It reached its lowest point about 3 sec. after the last contraction of the reticulum. The frequency of the cycles of pressure changes in the reticulum and omasum varied considerably, being fastest during eating (up to 105/hr.) and slowest during rumination (typically, about fifty/hr.).

The reticulo-omasal orifice remained loosely open for 60–70 % of the time occupied by the co-ordinated cycles of pressure changes in the reticulo-rumen and omasum. In each cycle there was, during resting, an increase in pressure in the orifice at the time of the first reticular contraction. During rumination this increase was preceded by a similar increase in pressure at the time of the extra reticular contraction. At the time of the last reticular contraction pressure in the orifice fell to a point on, but never below, the base-line recorded between cycles of activity.

The greatest differential pressure between the reticulum and the abomasum occurred at the time of the reticular contraction and was probably about 1 cm. mercury. At this

time a flow of digesta might be expected. The rate of such a flow has been expressed on theoretical considerations as a function of the differential pressure, and the resistance between the two regions (Werle, Brody, Ligon, Read & Quigley, 1940-1)

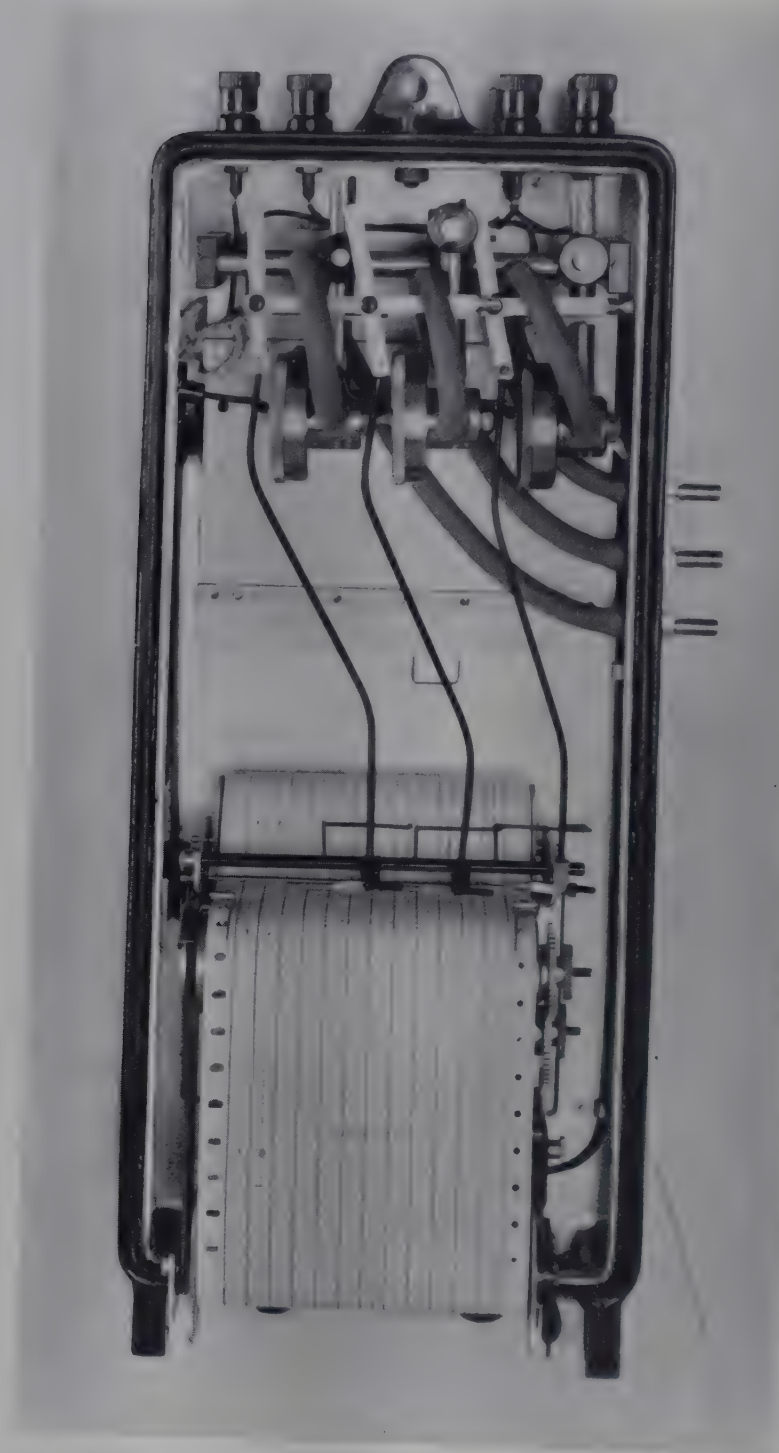
$$\text{Rate of flow} = K \times \frac{\text{pressure } A - \text{pressure } B}{\text{resistance to flow}},$$

where A = pressure in compartment being evacuated and B = pressure in compartment into which food is flowing.

In the cow the distance between the reticulo-omasal and omaso-abomasal orifice is about 10 cm. (Ellenberger & Baum, 1926). It is not clear whether the passage from the reticulum to the omasum should be considered a one-stage process with the digesta in the neck of the omasum providing merely a resistance to the flow, or whether it should be considered a two-stage process, with the digesta in the omasum, and possibly a contraction in the region of the bridge of the omasum (Wester, 1926) preventing an uninterrupted flow. In either instance it is likely that the transfer of digesta would occur at the time of the last reticular contraction and would be a continuous process occurring most rapidly during eating.

In view of the solid nature of digesta in the neck of the omasum, as found by palpation through the reticulo-omasal orifice, it seems likely that the transfer of digesta from the reticulum to the abomasum was in two stages, controlled by a valve-like action of the omasum. We feel that in further investigations on this point the following hypothesis should be borne in mind. During each cycle of movement the first rise in pressure in the omasum may have represented a constriction of digesta in the neck, and possibly a movement of digesta onward to the abomasum. At the time of the fall in pressure in the neck of the omasum, semi-liquid digesta may have entered from the reticulum and with the resumption of high pressure in the omasum there may have been a transfer of digesta to the abomasum, backflow to the reticulum being prevented by the closed orifice. It also seems likely that, during the periods of high pressure in the neck of the omasum, liquid, and not solid as suggested by Wester (1926), would tend to be squeezed between the leaves of the omasum as if the digesta were pressed against a sponge. The particles of solid which are undoubtedly to be found on post-mortem examination between the leaves may represent only the particles that would inevitably be carried by accident with the liquid. The contents of the omasum represent only 7-8% of the contents of the whole stomach (Schalk & Amadon, 1928); if, therefore, all digesta were to pass between the leaves, such passage would need to be very rapid, but the fact that no mechanism by which digesta may be expelled from between the leaves has yet been described suggests that there is no such rapid expulsion and that much of the solid digesta does not pass between the leaves.

The reticulum is the pace-maker for the cycle of movements which involves the reticulo-rumen. Recordings taken during this study showed that some part of the omasum invariably began to contract before the reticulum, suggesting that the omasum may have been the pace-maker for the reticulum. Further support for this theory is provided by an observation by Wester (1926), that the contractions of the reticulo-rumen could be stopped by applying pressure to the bridge of the omasum.



If a cow were receiving a diet containing 10 kg. dry matter daily, of which 60 % was digested, and if 50 % of the total digestion of dry matter occurred in the reticulo-rumen, then 7 kg. dry matter would have to pass through the reticulo-omasal orifice each day. If the mean rate of reticulum contraction were sixty/hr. the mean flow of dry matter through the orifice would be 4.6 g. per cycle of contraction. The mean dry-matter content of reticulum contents is about 6 % (Balch, 1950). It is likely, therefore, that about 80 ml. water would also pass through the orifice at each contraction, or more than 100 l./24 hr.

SUMMARY

1. A description is given of a simple type of kymograph suitable for taking tambour readings over periods of up to 24 hr.
2. The movements of the reticulo-omasal orifice in relation to the contractions of the reticulum, the anterior pillar of the rumen, the omasum and the abomasum were studied by means of small, lightly inflated balloons.
3. During resting, eating, ruminating, lying, drinking and while the cow was being milked, the reticulo-omasal orifice functioned to a constant pattern. For the greater part of the reticulo-ruminal cycle of contraction the reticulo-omasal orifice was loosely open, but following the last reticular contraction the orifice closed strongly.
4. The last reticular contraction was accompanied by a marked fall in pressure in the neck of the omasum, followed by a marked rise of pressure in the omasum which coincided with the closure of the reticulo-omasal orifice.
5. The transfer of digesta from the reticulum and anterior rumen was most likely to occur at the time of the last reticular contraction, coinciding with the fall in pressure in the omasum. It is therefore probable that the passage of digesta from the reticulo-rumen is relatively continuous throughout the 24 hr. and is largely controlled by the valve-like action of the omasum.

We wish to thank Dr S. Bartlett for his support of this work and for arranging that two of us (A.K. and G.H.) should do it while visiting this Institute. We are also much indebted to Dr A. T. Phillipson of the Rowett Research Institute who read the manuscript and offered most valuable suggestions. We also wish to thank Messrs Evershed and Vignoles Ltd., Acton Lane, Chiswick, for their advice on the choice of the recorder and Mr H. S. Hall and his staff in the Engineering Department of this Institute for carrying out the modifications. We are grateful to Mr N. Gruber for his care in photographing the apparatus.

EXPLANATION OF PLATE

- Pl. 1. Front view of the instrument for recording pressure changes in the stomach of a cow. Three tambours are clamped to a crossbar at the top. The compensating weight on a projection from the writing arm can be seen clearly in the left-hand tambour. The paper moves forwards and downwards. In this view the lid of the instrument has been removed.

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Excretion of Vitamin C in Urine Following Repeated Administration of Big Test Doses

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The vitamin C saturation test as introduced by Harris & Ray (1935) is based on counting the number of days necessary for administration of standard test doses of vitamin C before a large overflow of the vitamin appears in the urine, this number increasing in proportion to the lowness of the past intake. For convenience in examining large groups of subjects, a simplified procedure, involving the collection of a single specimen of urine each day during the peak of excretion rather than of the whole day's output, was recommended by Harris & Abbasy (1937) as sufficiently accurate for routine surveys. Thus modified, the saturation test is easily applicable to group surveys and has been found capable of distinguishing between groups of children or adults at slightly different levels of intake (e.g. Harris, 1940, 1943; Atkins, 1943). The procedure adopted by various workers has, however, differed somewhat, not only as to the size of the test dose but also in choice of excretion period.

In carrying out a small-scale survey using the simplified saturation test as outlined by Harris & Abbasy (1937), it was thought that some supplementary tests, involving the estimation at graded intervals of the total 24 hr. output, might be of interest. This would give some idea of the relation in time between the peak of excretion on approaching saturation and the test period selected (e.g. a 3 hr. period, from the 4th to the 7th hour after the test dose); at the same time information might be obtained about the reliability of this short-period excretion as a criterion of the total overflow.

EXPERIMENTAL

Subjects and their treatment. Five male students at a residential college, 20-21 years of age were given big test doses of ascorbic acid by mouth on each of 4 consecutive days (6-9 June 1950).

The test doses amounted to 10 mg./kg. body-weight and were taken in the morning except on the 1st day when they were taken at noon. Immediately before receiving the test dose the subject emptied his bladder; subsequently urine was collected at frequent intervals and analysed for vitamin C. All the subjects had undergone saturation tests about 4 months before. The dietary intakes of vitamin C were estimated to be about 20 mg./day, and serum values below 0.2 mg. vitamin C/100 ml. were found in all cases when tested just before administering the first dose.

Ascorbic-acid estimation. The ascorbic acid in the urine was estimated by the indophenol method, the reduction of the dye being determined by using a photo-electric colorimeter (Lumetron, Photovolt Corporation, New York City) instead of by titration. It was not thought necessary to correct for the blank (that is any non-specific indophenol-reducing substances).

The following procedure was used. To a measured quantity of urine (varying from 1 to 8 ml., according to a preliminary rough estimation of the concentration of the ascorbic acid) were added 2 ml. of a 20 % (w/v) solution of trichloroacetic acid, and the volume was then made up to 10 ml. with distilled water. Of the urine so diluted a suitable portion, not exceeding 2 ml., was transferred to each of a matched pair of test-tubes containing 4 ml. of an acetic acid-sodium acetate buffer of pH about 4.1. When less than 2 ml. were used, sufficient 4 % (w/v) solution of trichloroacetic acid was added to make the volume up to 6 ml. One of the tubes, after the addition of 2 ml. water, was used for zero adjustment of the colorimeter (density scale, green filter 530), and 2 ml. of a solution of dichlorophenolindophenol were then added to the other: the density reading was next taken as rapidly as possible. The difference between this reading and that of the dye control (read against water as the blank) is a measure of the extent of reduction of the dye, the dye control having been put up in the same way except that the diluted urine was replaced by an equal amount of the 4 % trichloroacetic-acid solution. The concentration of the dye solution was such as to give a reading of 5 % on the transmission scale. The dye control then gave a reading of about 2.7 on the density scale.

By this method readings can be taken rapidly, within 5–10 sec., so as to minimize the interfering action in normal urine of reducing substances other than ascorbic acid. When normal urine with low ascorbic-acid concentration (less than 1 mg./100 ml.) was tested by this method, it was found that the amount of reduction might increase considerably within the first 2 min. or so—the time often required for titration. At higher concentrations, when only small amounts of urine, diluted to one-fifth or one-tenth, were used in the test, the increase in reduction after the first reading was, however, negligible.

RESULTS

The values found for vitamin C in urine collected at intervals after the test doses are given in Table 1. Fig. 1 shows the cumulative excretion of each successive day.

On the 1st day the excretion was low in all subjects, varying from 8.7 to 19.2 mg. in the 20½ hr. Already on the 2nd day three of the subjects gave a marked response. Their response was considerably greater on the following day, but after the fourth test dose

Table I. Excretion of ascorbic acid in the urine of five subjects following the administration of one test dose (10 mg./kg. body-weight) on each of 4 consecutive days

Subject																
Period (hr. after dose)	Length of period (hr.)	H.G. (65 kg.)			J.A. (80 kg.)			K.S. (69 kg.)			V.P. (77 kg.)			G.I. (74 kg.)		
		Ascorbic acid			Ascorbic acid			Ascorbic acid			Ascorbic acid			Ascorbic acid		
		Urine (ml.)	mg./100 ml. urine	mg.	Urine (ml.)	mg./100 ml. urine	mg.	Urine (ml.)	mg./100 ml. urine	mg.	Urine (ml.)	mg./100 ml. urine	mg.	Urine (ml.)	mg./100 ml. urine	mg.
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
				0.43			0.43			0.18			0.54			
				—			—			—			—			—
				—			—			—			—			—
0-4	4	584	0.57	3.33	80	3.39	2.71	194	4.64	9.00	334	1.61	5.38	250	0.71	1.78
4-7	3	106	3.21	3.40	88	3.03	2.67	60	4.11	2.47	544	0.76	4.13	115	2.14	2.46
7-10	3	270	1.43	3.86	130	2.14	2.78	168	1.43	2.40	208	1.25	2.60	157	1.79	2.81
10-20½	10½	555	0.54	3.00	770	0.53	4.08	500	1.07	5.35	440	0.46	2.02	307	0.54	1.66
Total	20½	1515	—	13.59	1068	—	12.24	922	—	19.22	1526	—	14.13	829	—	8.71
				—			—			—			—			—
				—			—			—			—			—
				—			—			—			—			—
				—			—			—			—			—
0-2	2	180	14.3	25.7	205	1.4	2.9	85	11.4	9.7	144	1.7	2.4	167	0.9	1.5
2-4	2	157	22.6	35.5	120	2.1	2.5	85	37.5	31.9	160	30.4	48.6	75	1.1	0.8
4-7	3	149	6.0	8.9	169	1.9	3.2	200	13.1	26.2	220	2.9	6.4	193	0.9	1.7
7-9	2	145	6.8	9.9	94	3.4	3.2	95	5.3	5.0	245	4.3	10.5	120	1.3	1.6
9-11	2	85	3.2	2.7	48	3.6	1.7	67	3.2	2.1	175	1.1	1.9	89	2.3	2.0
11-13	2	133	1.8	2.4	90	1.8	1.6	130	1.8	2.3	500	0.14	0.7	100	0.9	0.9
13-24	11	710	0.4	2.6	560	0.9	5.0	365	0.93	3.4	540	0.54	2.9	360	0.50	1.8
Total	24	1559	—	87.7	1286	—	20.1	1027	—	80.6	1984	—	73.4	1104	—	10.3
				—			—			—			—			—
				—			—			—			—			—
				—			—			—			—			—
				—			—			—			—			—
0-2	2	204	7.1	14.5	64	1.4	0.9	133	19.3	25.7	125	12.1	15.1	120	1.5	1.8
2-4	2	190	42.9	81.5	120	53.6	64.3	156	89.3	139.3	172	96.4	165.8	250	12.9	32.3
4-7	3	617	25.0	154.3	212	53.6	113.6	520	24.3	126.4	542	25.7	139.3	384	17.9	68.7
7-9	2	170	13.6	23.1	90	8.6	7.7	146	16.9	24.7	327	4.8	15.7	115	15.7	18.1
9-11	2	205	8.6	17.6	130	8.6	11.1	158	12.9	20.4	135	3.6	4.9	223	1.0	2.2
11-13	2	222	5.7	12.7	82	4.3	3.5	202	7.9	16.0	193	7.5	14.5	120	2.1	2.5
13-23	10	685	1.1	7.5	602	2.1	12.6	460	3.5	16.1	275	3.6	9.9	655	0.43	2.8
Total	23	2293	—	311.2	1300	—	213.7	1775	—	368.6	1769	—	365.2	1867	—	128.4
				—			—			—			—			—
				—			—			—			—			—
				—			—			—			—			—
				—			—			—			—			—
0-2	2	710	28.6	203.1	90	19.6	17.6	585	7.9	46.2	80	150.0	120.0	120	9.3	11.2
2-4	2	213	78.6	167.4	90	28.6	25.7	122	114.3	139.4	370	26.1	96.6	110	150.0	165.0
4-7	3	100	14.3	14.3	190	50.0	95.0	255	35.7	91.0	116	35.7	41.4	105	152.9	160.5
7-9	2	72	14.7	10.6	103	45.7	47.0	77	32.1	24.7	80	21.4	17.1	70	46.4	32.5
9-11	2	100	14.3	14.3	103	45.7	47.0	77	32.1	24.7	80	21.4	17.1	70	46.4	32.5
Total	10	1000	100.0	100.0	1000	100.0	100.0	1000	100.0	100.0	1000	100.0	100.0	1000	100.0	100.0

only one of them still showed some further increase in excretion, but the other two showed a slight decline. Two of the subjects showed almost no response until after the third test dose. It was not found worth while to calculate all the values to a standard weight (70 kg.) as the main conclusions arrived at would obviously not have been thereby affected.

Table 2 shows the amounts of vitamin C excreted at certain periods on the 2nd and 3rd days, as percentages of the total output in 24 or 23 hr., respectively.

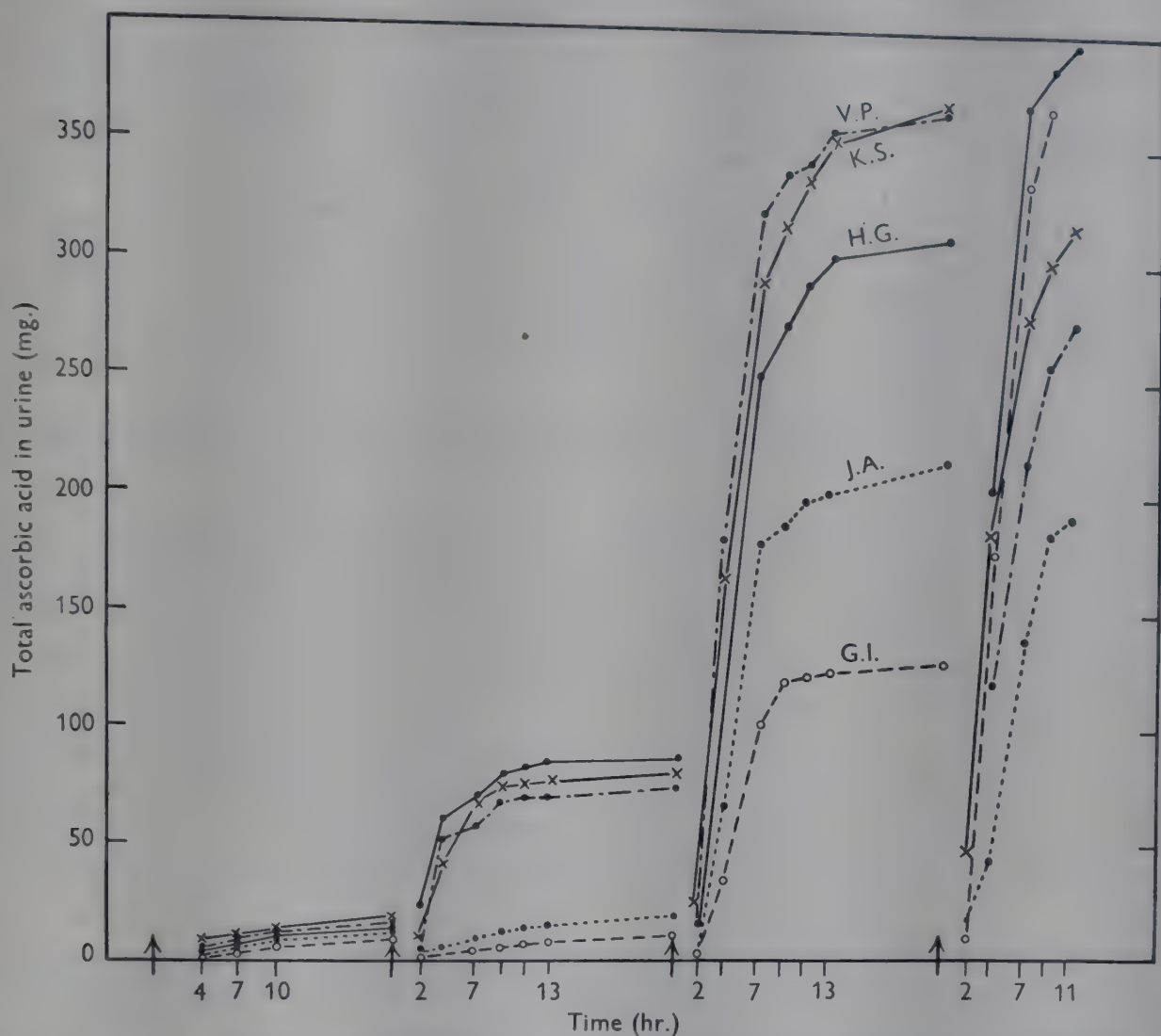


Fig. 1. Cumulative daily excretion of ascorbic acid in the urine of five male students 20–21 years old, following the administration of big test doses (10 mg./kg. body-weight) on each of 4 consecutive days, as indicated by the arrows (cf. also Table 1).

DISCUSSION

From Table 2 it is seen that when a marked response is reached (in three subjects on the 2nd day and in all on the 3rd day) a remarkably constant proportion, 94.1–97.8 %, of the 23–24 hr. output is excreted within the first 13 hr. following administration of the test dose. Also, the percentage excreted in the first 7 hr. is relatively constant, ranging from 78.2 to 87.7. With shorter periods, from the 2nd to the 4th and from the 4th to the 7th hr. after the test dose, however, this percentage varies considerably, especially during the initial response, and as often as not more was excreted in the first than in the second period. But, with one exception, both these periods together

Table 2. *Excretion of ascorbic acid in the urine of five subjects at certain intervals after the test dose (see Table 1), expressed as a percentage of the total output in 23 or 24 hr.*

Subject	Total output		Percentage of total output excreted in interval after dose			
	mg.	As percentage of dose				
			2-4 hr.	4-7 hr.	0-7 hr.	0-13 hr.
			2nd day, 24 hr.			
J.A.	20.1	2.5	12.4	15.9	42.8	75.1
G.I.	10.3	1.4	7.8	16.5	38.8	82.5
H.G.	87.7	13.5	40.5	10.1	79.9	97.0
K.S.	80.6	11.7	39.6	32.5	84.1	95.8
V.P.	73.4	9.5	66.2	8.7	78.2	96.0
3rd day, 23 hr.						
J.A.	213.7	26.7	30.1	53.2	83.7	94.1
G.I.	128.4	17.4	25.2	53.5	80.1	97.8
H.G.	311.2	47.9	26.2	49.6	80.4	97.6
K.S.	368.6	53.4	37.8	34.3	79.1	95.6
V.P.	365.2	47.4	45.4	38.1	87.7	97.3

(i.e. from the 2nd to the 7th hr. after the test dose) represent a fairly constant proportion of the total output.

From this limited number of observations it would appear that the excretion during the period from 4 to 7 hr. after the test dose is not a good criterion of the magnitude of the response, as too often it does not cover the peak of excretion. Thus in two subjects (H.G. and V.P., Table 1 and Fig. 1) an initial response occurred on the 2nd day, but would hardly have been recognized if only the 4th to 7th hr. specimen had been tested. It should, however, be noted here that, although, as mentioned above, this period was originally recommended by Harris & Abbasy for the collection of the test specimen, Harris (1940, 1943) later preferred a $2\frac{1}{4}$ hr. sample taken between $3\frac{1}{2}$ and $5\frac{3}{4}$ hr. after the test dose (i.e. around the 4th and 5th hr.), when he found the excretion to be at its peak.

The data contained in Table 1 relating to thirteen instances of marked response do not allow an exact timing of the peak of excretion, but obviously it has (with possibly one exception, H.G. on the 2nd day) occurred well within the range of the 2nd to the 7th hr. after the test dose and probably in all but two instances (H.G., 2nd day and J.A., 4th day) within the period from 3 to 6 hr., or even from 3 to $5\frac{1}{2}$ hr., after the test dose.

Only two of the students showed continued increase in excretion up to the 4th day; the other three apparently excreted slightly less on the 4th day than on the 3rd. Unfortunately circumstances did not allow the experiment to be prolonged for some days more so as to see whether this apparent fall in excretion was of any significance. Shortly afterwards, however, similar tests were carried out with one adult male receiving a test dose of 750 mg. (10 mg./kg. body-weight) for 8 consecutive days. Fig. 2 shows the amounts of vitamin C excreted on each of these days, except the 6th when only the night specimen of urine was available. Judging from this specimen it appeared that on the 6th day the excretion was already diminishing. Anyhow, there

was an appreciable, progressive fall for the next 2 days, in spite of continued test dosing as before. After the administration of test doses was discontinued the expected abrupt fall to a still lower level of excretion took place.

A corresponding fluctuation in the test-period excretion, in spite of continued dosage after saturation has apparently been reached, has often been observed by earlier authors (e.g. Harris, 1940, 1943). The most reasonable explanation seems to be that repeated intake of excessive amounts of ascorbic acid leads to an increased rate of destruction.

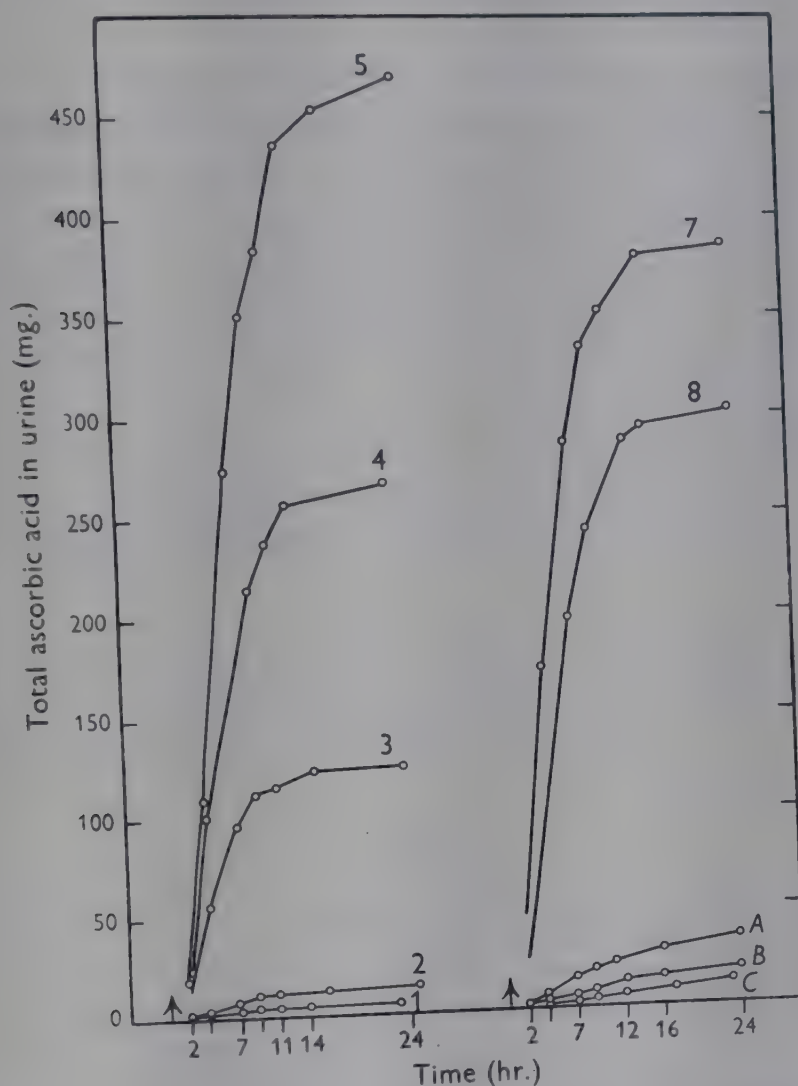


Fig. 2. Cumulative daily excretion of ascorbic acid in the urine of one adult male (weight 75 kg.) following the administration of a 750 mg. test dose on each of 8 consecutive days. (The curve for the 6th day is not shown.) The curves A, B and C represent the excretion on the first 3 days after discontinuing the test dosing.

The maximum output, as observed in these experiments (Tables 1 and 2, Fig. 1) when saturation is reached, is roughly about 50-60 % of the test dose, and—as far as can be judged from the 2-3 hr. afternoon samples—this seems to be in good accord with the findings of Harris (1940, 1942), and others who have used test doses of similar size. When smaller doses are given the percentage excreted may, however, be higher (Spellberg & Keeton, 1939).

Apart from losses through destruction in the tissues, it is possible that appreciable amounts of these unphysiologically large test doses are destroyed before absorption in

the intestine. Whether or not destruction in the tissues is as great before as after saturation has been reached, it may be expected that the total losses are considerable. It therefore appears obvious that the vitamin C deficit cannot be exactly expressed quantitatively in terms of the ascorbic acid required to induce urinary response.

SUMMARY

1. Five male students were given big test doses of ascorbic acid (10 mg./kg. body-weight) on 4 consecutive days. Specimens of urine were collected at frequent intervals and analysed for ascorbic acid.

2. When a distinct response had been obtained, it was found that 94–98 % of the 23–24 hr. output of ascorbic acid were excreted in the first 13 hr., and 78–88 % in the first 7 hr. after administration of the test dose. In shorter periods within the first 7 hr. the relative amount excreted was more variable. As often as not, more was excreted in the 2 hr. period between 2 and 4 hr. after the dosage than in the following 3 hr. period. A distinct response shown by two of the subjects on the 2nd day would hardly have been noticed if the 3 hr. specimen only, collected between the 4th and 7th hr. after the test dose, had been examined.

3. Not more than about 50–60 % of the ascorbic acid administered could be recovered in the urine when full saturation was reached. This confirms previous findings that considerable losses occur through destruction or possibly through incomplete absorption of the large doses.

My thanks and appreciation are due to Mr Björn Jakobsson and Mr Bjarni Bjarnason, headmasters of the schools at Laugarvatn, for the facilities granted for the carrying out of this investigation and to the students for their excellent and conscientious co-operation.

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The Apparent Intestinal Synthesis of Carotene by Sheep*

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During an investigation into certain aspects of the carotene metabolism of ruminants, it was desired to obtain an indication of the effect of the composition of the feed on the availability of carotene to the animals. The most convenient method of measuring approximately the carotene absorption appeared to be by determining its apparent digestibility using the ordinary digestibility-trial technique. However, if any appreciable decomposition occurred in the digestive tract, the calculated digestibility might bear no relationship to the actual absorption. It seemed desirable, therefore, to verify whether there is any oxidative decomposition in regions where absorption of carotene does not occur by determining carotene content, relative to an inert reference substance, at various points through the digestive tract. In addition, it was anticipated that the same experiment would afford some explanation of an apparently anomalous excretion of carotene observed in preliminary digestibility trials, where some of the sheep were found to be excreting more carotene than they were consuming, excretion in some cases reaching 160 % of the ingested provitamin.

EXPERIMENTAL

Lignin as reference substance. Lignin was selected as a suitable reference substance for these experiments, and a digestibility trial with pasture-fed sheep provided an opportunity for checking the recovery of lignin from grass. At the same time, the excretion of carotene was further investigated by estimating carotene:lignin ratios in samples of faeces collected at intervals from these animals.

Estimation of carotene and lignin. Carotene was estimated in feed, ingesta and faeces by a modification (McGillivray, 1950) of the cold-extraction method using a 'foaming mixture' of light petroleum and ethanol as described by Moore & Ely (1941). The method of Ellis, Matrone & Maynard (1946) was used for the lignin determinations. All assays were carried out in duplicate and, in order to reduce sampling errors, the residue from the carotene determinations was dried at room temperature, ground, and used for the lignin estimations. Carotene:lignin ratios were calculated in all instances in mg. carotene/g. lignin. The reproducibility of these ratios was investigated in a number of instances, a typical result, where six determinations were made on a well-mixed sample of dried grass being, carotene 355 (± 15) mg./kg. and lignin 48 (± 2.0) g./kg. giving a carotene:lignin ratio of 7.4 with a standard deviation of ± 0.4 or 5.5 %.

* This investigation forms part of a thesis submitted for the degree of Ph.D. of the University of New Zealand.

Differences of the same order were found between determinations on samples of ingesta and faeces.

Carotene ratios through the digestive tract. Samples of ingesta, each equivalent to about 5 g. of dry matter, were collected from the four stomachs, from various points along the small intestine and from the caecum, colon and rectum of a pasture-fed sheep immediately after slaughter. These, together with a sample of freshly voided faeces and a sample, as representative as possible, of the pasture on which the animal had been grazing, were assayed for carotene and lignin. Similar determinations were made on three other sheep, samples on one occasion being taken at more frequent intervals through the intestine.

Carotene excretion and lignin recoveries. The level and uniformity of carotene excretion by sheep was investigated by determining carotene:lignin ratios in samples of faeces collected twice daily from four pasture-fed animals which formed part of a group used for a digestibility trial. For the trial, the faeces were collected by the conventional bag method and, when these bags were emptied, samples (about 25 g. each) of the most recently voided faeces were collected. These, together with representative pasture samples, were held at 0° until the end of the trial, when they were assayed in duplicate for carotene and lignin. The digestibility of lignin was calculated in the usual way from the trial figures. As a further check on the constancy of its composition, the nitrogen contents of samples of lignin extracted from pasture, caecal contents and faeces were also determined.

RESULTS

The variations in carotene:lignin ratios found in the first sheep investigated are shown in Table 1. These figures show a decrease in the carotene ratios to a minimum in the jejunum, and then an increase reaching a maximum in the caecum followed by a small decrease through the colon and rectum. Table 2 shows the ratios found in the second animal, where particular attention was paid to the changes through the intestine. The trends, which were similar to those observed in the first animal, were also apparent in the other two sheep examined, the carotene:lignin ratios decreasing from 16.7 in the pasture to a minimum of 13.7 in one case, and 15.6 in the other, in the mid-jejunum and increasing to maxima of 17.3 and 20.0, respectively, in the caecum. With the first

Table 1. *Carotene:lignin ratios in grass and through the digestive tract of a pasture-fed sheep*

Origin of sample	Ratio (mg. carotene/ g. lignin)	Origin of sample	Ratio (mg. carotene/ g. lignin)
Grass	21.2	Ileum:	
Rumen	12.3	mid 2 ft.	23.4
Reticulum	16.7	last 2 ft.	25.5
Omasum	14.0	Caecum	28.1
Abomasum	22.0	Colon	27.0
Duodenum	22.8	Rectum	21.4
Jejunum:		Faeces	22.5
first 6 ft.	23.3		
mid 6 ft.	15.2		
last 6 ft.	15.9		

animal the ratios varied considerably through the four stomachs owing, possibly, to the retention of more fibrous material of low carotene content in the rumen and omasum. In all instances, however, good agreement was found between the ratios in the pasture and in the abomasum, indicating little loss of carotene during passage through the stomachs.

Table 2. *Carotene:lignin ratios through the digestive tract of another sheep*

Origin of sample	Ratio (mg. carotene/g. lignin)	Origin of sample	Ratio (mg. carotene/g. lignin)
Grass	16.7	Small intestine:	
Abomasum	14.9	tenth 6 ft.	22.2
Duodenum	15.5	eleventh 6 ft.	18.8
Small intestine:		twelfth 6 ft.	17.6
first 6 ft.	11.7	Caecum	22.2
second 6 ft.	9.0	Colon:	
third 6 ft.	9.9	first 2 ft.	21.8
fourth 6 ft.	12.0	second 2 ft.	21.0
fifth 6 ft.	13.9	third 2 ft.	20.1
sixth 6 ft.	13.4	fourth 2 ft.	20.4
seventh 6 ft.	16.0	fifth 2 ft.	18.7
eighth 6 ft.	13.8	sixth 2 ft.	18.9
ninth 6 ft.	16.6	Faeces	17.5

The excretion of carotene by the four pasture-fed sheep was fairly uniform for each animal over the 3-day collection period but varied somewhat between animals. The average carotene:lignin ratio in the pasture was 18.5 and the ratios found in the faeces are shown in Table 3. The percentage carotene excreted was high in all cases, animal no. 2 showing a marked negative balance. The average lignin recovery was 96.0 %, indicating negligible digestibility. No significant differences were found in the nitrogen content of the lignin isolated from pasture, caecal contents and faeces, all samples containing 1.8–2.5 % nitrogen.

Table 3. *Carotene:lignin ratios in faeces of four sheep*

Day	Sheep no.			
	1	2	3	4
1st: a.m.	15.7	22.4	17.0	16.0
p.m.	13.4	19.8	18.6	18.8
2nd: a.m.	12.2	21.8	18.2	21.1
p.m.	15.4	21.5	17.9	17.9
3rd: a.m.	15.4	20.3	16.5	16.3
Average	14.4	21.2	17.6	18.0
Carotene excreted (as percentage of that ingested)	78.0	114.5	95.2	97.4

DISCUSSION

It seems reasonable to assume that carotene absorption would, at least in part, account for the decrease in carotene:lignin ratios through the upper portions of the small intestine, and that the decrease through the colon and rectum might be attributed to oxidative decomposition of the pigment. The increase in the ileum and caecum is more

difficult to explain, but could result from (a) a partial digestion of the lignin, (b) the formation in the lower intestine of a pigment not separated from carotene on the Hyflo Super-Cel (Fisher Scientific Company and Eimer and Amend, New York) chromatographic column, or (c) a synthesis of carotene in the intestine. Ellis *et al.* (1946) have shown that the lignin estimated by their method represents an almost completely indigestible fraction of herbage. This, supported by the 96 % recovery of ingested lignin from faeces in this investigation, excludes the possibility of the increase in carotene:lignin ratios in the caecum being due to the use of lignin as an inert reference substance. This conclusion is also supported by the constancy of the nitrogen content of the lignin samples isolated from various sources. Although the lignin molecule is believed not to contain nitrogen, it has not been possible to isolate nitrogen-free lignin from succulent plant tissues. If, as is generally considered (MacDougall & DeLong, 1948*a-c*), this nitrogen is due to the condensation of protein molecules with the lignin, possibly during extraction, even small changes in the nitrogen content would have markedly affected the carotene ratios.

An extensive rechecking and investigation of the accuracy of the method used for the estimation of carotene (McGillivray, 1950) eliminated the possibility of appreciable errors in the carotene assays. Although the possible presence in faeces and other materials of a yellow pigment or artifact that cannot be separated from carotene by the normal phasic methods is well recognized (Booth, 1945), it should be possible to effect a separation by chromatographic methods. However, repeated chromatographing on columns of Hyflo Super-Cel or of magnesium oxide and Hyflo Super-Cel failed to reveal the presence of pigments other than the carotenes. The identity of the pigment obtained from caecal contents was further confirmed by a comparison of its absorption spectrum with that of a sample of carotene extracted from fresh pasture. As shown in Fig. 1 the pigment from the intestine appears to be identical with the carotene in the pasture, allowing for some isomerization in the digestive tract.

It appears, therefore, that some synthesis of carotene must have occurred in the digestive tracts of the sheep. Negative balances of carotene have been reported previously (e.g. by Whitnah, Peterson, Atkeson & Cave, 1939) but in all such instances phasic separation methods had been used for separating the carotenes from other pigments. Since these methods are not specific for the carotenes, subsequent workers have apparently attributed the findings entirely to the presence in the carotene fraction of other epiphasic pigments. The presence of these pigments, which form a yellow band immediately above the carotenes on the chromatographic columns, has been noted in this investigation. The quantities have, however, been small, representing less than 15 % of the total epiphasic pigments present, and it seems possible that carotene synthesis might also have contributed to the negative digestibilities previously reported.

Several micro-organisms, e.g. *Staphylococcus aureus*, are capable of synthesizing carotenes (Zechmeister & Cholnoky, 1943, pp. 126, 137), and the possibility of intestinal synthesis by these organisms has apparently been considered previously, but in a recent report it was concluded that formation of carotene did not occur in the digestive tract of man (Hume & Krebs, 1949). If micro-organisms are responsible for the synthesis in sheep it should be possible to show an increase in carotene content on incubating

caecal or ileal contents. Although this aspect of the work is still under investigation, it has not so far been possible conclusively to demonstrate carotene formation under these conditions, but synthesis has been shown to occur on a solid medium containing agar 2 %, tryptose 2 %, dextrose 0.1 %, and sodium chloride 0.5 %, inoculated with caecal contents collected under aseptic conditions. Carotene, identified by its absorption spectrum, was formed in quantities equivalent to 1.2–1.8 $\mu\text{g./ml.}$ medium. No attempt has been made to identify the micro-organisms responsible.

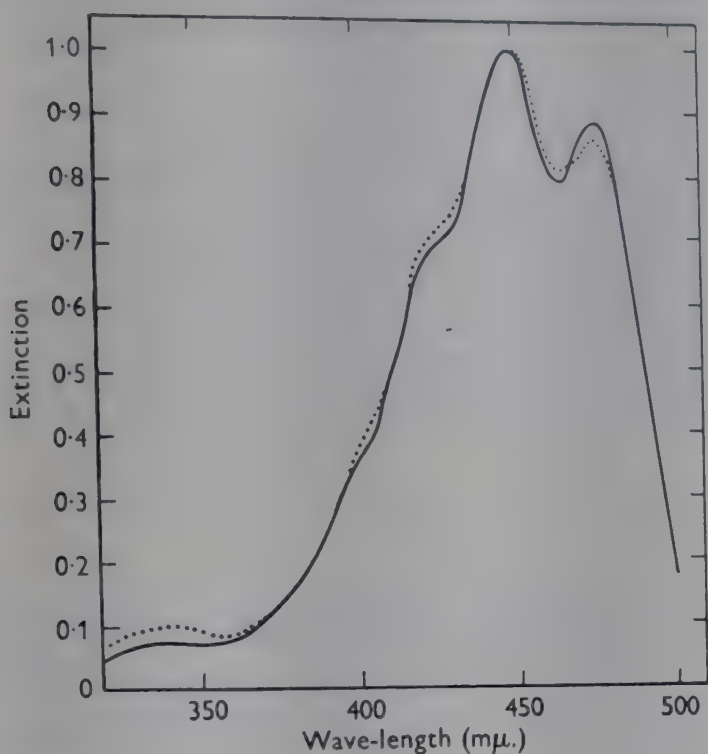


Fig. 1. Absorption spectrum of carotene. Isolated from: —, grass;, caecal contents of a sheep.

Apart from this synthesis of carotene, marked decomposition of the provitamin appears to occur in the colon and rectum, so that the apparent digestibility, as calculated from the difference between the amounts ingested and excreted, gives no indication of actual absorption. A point of immediate interest is whether the synthesized carotene can be utilized by the animal. No absorption of carotene or vitamin A occurs in the caecum or colon (Barrick, Andrews & Bullard, 1948) but it is possible that some absorption occurs in the lower portions of the ileum.

SUMMARY

1. Carotene:lignin ratios were determined at different points through the digestive tracts of four sheep. The ratios decreased through the upper portions of the small intestine, increased through the ileum reaching a maximum in the caecum, and decreased slightly through the colon and rectum.

2. The increase in carotene:lignin ratio was not due to a partial digestibility of the lignin fraction of the herbage or to the erroneous estimation as carotene of some non-carotene pigment formed in the intestine.

3. It is suggested that carotene is synthesized by the micro-organisms of the ileum and caecum. Such synthesis of carotene by intestinal micro-organisms has been demonstrated on an agar medium inoculated with caecal contents.

The interest and helpful advice of Dr C. R. Barnicoat, Head of the Biochemistry Department during the course of this investigation, is gratefully acknowledged. The work was carried out during the tenure of a University Research Fellowship and financed by University Research Grants.

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The Composition of Human Milk with Special Reference to the Relation between Phosphorus Partition and Phosphatase and to the Partition of Certain Vitamins

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Two of the present authors are making a detailed study of the composition of the milk and colostrum of cows and goats, as affected by hormonal and nutritional influences. The results, which will be published shortly, showed that ester phosphorus was negatively correlated with phosphatase, the coefficient of correlation being -0.95 . In cows treated with thyroxine or thiouracil the same correlation obtained (Chanda & Owen, 1949). Lipid phosphorus and phosphatase were similarly correlated. During the change from colostrum to milk the correlations were still found. There was likewise (Chanda, McNaught & Owen, 1949) a negative correlation between phosphatase and phosphorylated vitamin B₁ in milk, as previously reported by Houston, Kon & Thompson (1940). The present work was undertaken to see whether the changes in human milk at the beginning of lactation were at all comparable with those in cow's

milk immediately after parturition or after hormone treatment. At the same time the partitions of carotenoids and of vitamin A were studied and some samples were tested for nicotinic acid.

During the recent war Kon & Mawson (1950) made a detailed study, reported to the Medical Research Council, of the changes in chemical composition of human milk throughout lactation. In their report, to which reference is again made in the discussion of the present results, nutritional influences on the composition of human milk were studied. The importance at present attached to natural variations in the composition of human milk is brought out also by Clements (1949).

EXPERIMENTAL

Collection of samples. One of us (B.C.) was responsible for collecting the milk and despatching it to the Hannah Institute for analysis. No milk was collected in the summer months to obviate the risk of possible changes in transit. Analyses were completed within 48 hr. of arrival of the samples at the laboratory. The total number of samples collected was fifty-two, but analyses for all the constituents studied could not be made on all of them. The number for which it was possible to make analyses is stated at the head of the relevant table.

Methods of analysis. Fats, solids-not-fat and protein were determined by the methods normally used for cow's milk at the Institute.

Phosphorus was partitioned by a slight modification of the method of Graham & Kay (1934). Trichloroacetic acid was cooled before use to avoid hydrolysis of phosphoric esters. The digestion of phosphorus compounds to phosphoric acid was carried out in micro-Kjeldahl flasks with concentrated H_2SO_4 and 30 % H_2O_2 (Horecker, Ma & Haas, 1940). Phosphoric acid was estimated by the method of Fiske & Subbarow (1925), with the aid of a Spekter absorptiometer.

Phosphatase could not be estimated by the short test of Kay & Graham (1935), and the long test (24 ± 2 hr. incubation), as modified by Neave (1939), was, therefore, used. The results are expressed arbitrarily as units which represent the number of mg. of phenol liberated from the British Drug Houses buffer-substrate tablets by the amount of enzyme in 100 ml. milk.

Vitamin B_1 was estimated in skimmed milk before and after treatment with takadiastase (Houston, *et al.* 1940). Takadiastase contains takaphosphatase (Kinnersley & Peters, 1938). Free vitamin B_1 was estimated by Jansen's method.

Nicotinic acid was estimated microbiologically by the method of Barton-Wright (1944) with *Lactobacillus arabinosus* 17/5. Acid production was determined by titration. Since the medium was brownish after incubation, a glass electrode and a Cambridge potentiometer were used to ensure titration to a constant pH.

In estimating carotenoids and vitamin A, it was found that besides β -carotene and xanthophyll, human milk fat contained appreciable amounts of α -carotene and lycopene. These substances were therefore separated chromatographically. The procedure for separating carotenoids, vitamin A ester and vitamin A alcohol was essentially that described by Ganguly, Kon & Thompson (1947). Lycopene, α -carotene and β -carotene were estimated as described below.

All the solvents used were purified. Diethyl ether was freed from peroxide by treatment with stannous chloride followed by distillation. Light petroleum and *n*-hexane were freed from aromatic hydrocarbons by treatment with H_2SO_4 (density 1.84 g./ml.), followed by distillation. Acetone was redistilled. Ethanol was freed from aldehydes by treatment with KOH and AgNO_3 followed by distillation just before use. The fat was extracted from the milk by a method based on that of Olson, Hegsted & Peterson (1939). To 50 ml. milk taken in a 250 ml. separating funnel, 7.5 ml. 35 % ammonia were added. After vigorous shaking the contents were allowed to stand for from 3 to 5 min., after which 30 ml. ethanol, 35 ml. freshly distilled diethyl ether and 15 ml. light petroleum (b.p. 40–60°) were added successively, the mixture being shaken after each addition. The separating funnel was then allowed to stand till the layers were cleanly separated. The lower layer was run off into a flask and the top layer collected in another flask. The lower layer was transferred back into the separating funnel and again extracted with 25 ml. diethyl ether and 10 ml. light petroleum. The two extracts were combined and allowed to stand in the separating funnel for 30 min. Any aqueous layer that separated was drawn off. After having been washed twice with 50 ml. portions of warm tap water with gentle shaking to avoid any formation of emulsion, the ether layer was dried over sodium sulphate and poured into a wide-mouthed flask. The sodium sulphate was washed twice with small volumes of ether which were added to the flask. The ether was removed by evaporation under reduced pressure on a water-bath at 70°. The fat so obtained was taken up in 5 ml. *n*-hexane and chromatographed in a 4 × 1 cm. column of alumina (Savory and Moore Ltd., London) previously moistened with *n*-hexane. α - and β -carotene, lycopene and vitamin A ester for the most part ran through with the fat. Addition of 20 ml. of 3 % acetone in *n*-hexane served to complete the elution but kept the vitamin A alcohol still on the column. The eluates of the same sample were combined (fraction 1). Vitamin A alcohol was then eluted with 20 ml. of 8 % ethanol in *n*-hexane, and the eluate was evaporated to dryness at 70° under reduced pressure. As soon as the solvent had all evaporated, the residue was taken up in 5 ml. *n*-hexane and its spectral absorption in ultraviolet light at 328 m μ . was determined with use of the correction of Morton & Stubbs (1946).

Fraction 1 was evaporated under reduced pressure, and the fat saponified with 1 g. KOH and 20 ml. alcohol by boiling for 5 min. The contents were diluted with three volumes of ice-cold water and the mixture was allowed to cool in the dark. The solution was then transferred to a separating funnel and extracted firstly with 50, then with 30 and finally with 20 ml. peroxide-free diethyl ether. The extracts were combined and washed with three 50 ml. lots of distilled water, shaking being gentle with the first and vigorous with the second and third lots. It was found necessary sometimes to use a little N-HCl in the second washing to remove the alkali completely. The final washing was tested to make sure that the extract was free from acid or alkali. The extract was dried over sodium sulphate, evaporated in a wide-mouthed flask under reduced pressure and then taken up in 5 ml. *n*-hexane before being chromatographed again on a 4 × 1 cm. column of alumina. α - and β -carotene were eluted with 3 % acetone in *n*-hexane. The eluate was made up to a suitable volume after evaporation at reduced pressure and the concentration of the carotenes was read in the spectrophotometer at 451 m μ .

with a tungsten lamp. Vitamin A ester of the original fat, now converted to vitamin A alcohol by the saponification, remained adsorbed on the column with the lycopene. The lycopene and vitamin A alcohol were eluted together by 10 % acetone in *n*-hexane. Lycopene was estimated in the spectrophotometer by its absorption at 505 $m\mu$. The value for $E_{1\text{cm}}^{1\%}$ in *n*-hexane was taken as 2000 (Morton, 1942).

It was not possible to estimate vitamin A in the same solution because the ultra-violet absorption of lycopene overlapped that of vitamin A. Fig. 1 shows that the

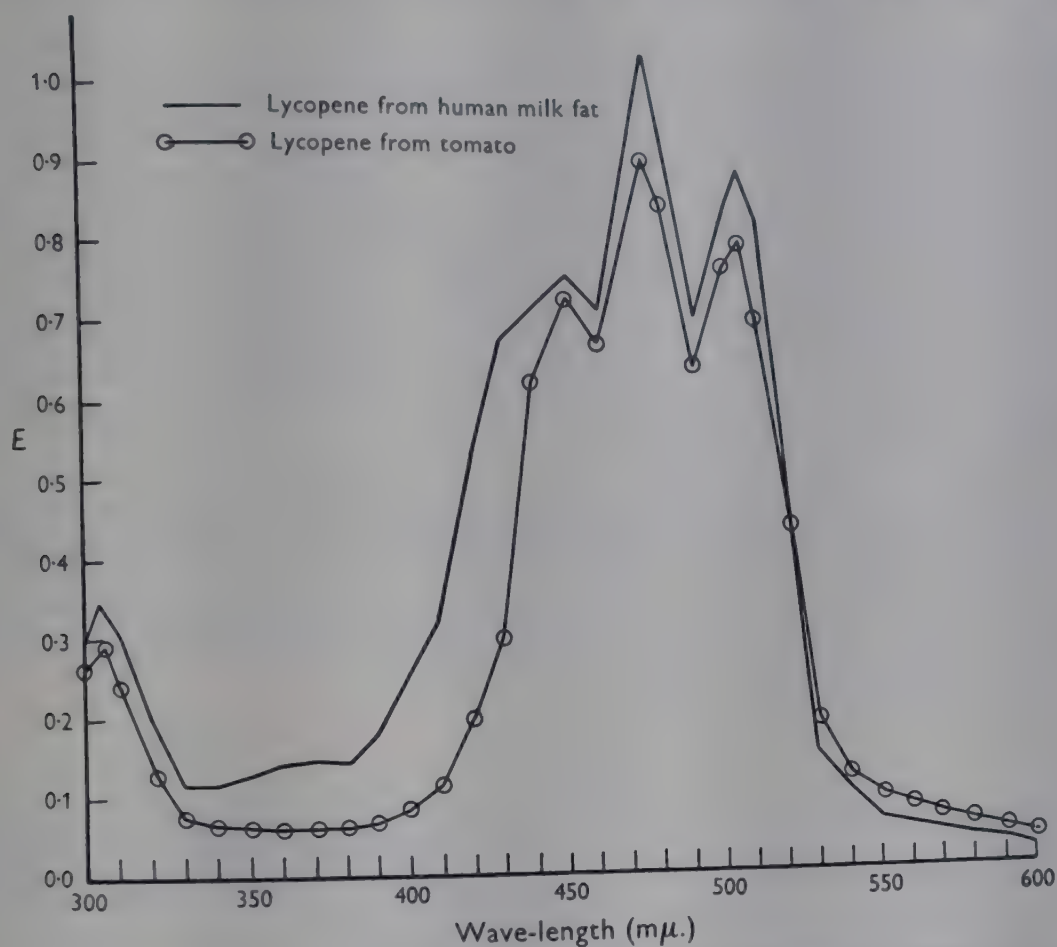


Fig. 1. Absorption spectrum of lycopene from tomato dissolved in *n*-hexane and of the red pigment from human milk. Both pigments were purified by adsorption chromatography on alumina.

absorption curve of lycopene has a distinct bend near 328 $m\mu$, so that the conditions defined by Morton & Stubbs (1946) for correction for irrelevant absorption did not obtain. Lycopene and vitamin A were, however, separated by the following methods. When fat containing vitamin A and carotenoids is chromatographed in *n*-hexane, lycopene can be held on the column of alumina, while the elution of carotene and vitamin A ester is completed with 3 % acetone in *n*-hexane, provided a sufficiently long column is used and the concentration of fat on the column is not too large. By adjusting the length of the column and decreasing the amount of material taken, the concentration of fat on the column is reduced, and vitamin A ester, free from lycopene, can be eluted. Accordingly, for the estimation of vitamin A ester a separate sample was extracted from the milk as already described, and chromatographed in two portions each in a 6×1 cm. column of alumina. By thus reducing the concentration of fat, the more slowly moving band of lycopene was retained, while α - and β -carotene and

vitamin A ester were eluted with the fat. Further elution of the column with 3 % acetone in *n*-hexane did not induce any appreciable movement of the lycopene band. The two eluates of the same sample, consisting of carotene and vitamin A ester, were combined and evaporated under reduced pressure. The fat was saponified. Carotene and vitamin A were extracted with ether as before and the final ether extract evaporated to dryness. The residue was taken up in 3 ml. *n*-hexane and chromatographed again. α - and β -carotene were eluted with 3 % acetone in *n*-hexane, and vitamin A ester, now converted to vitamin A alcohol, was eluted separately with 8 % ethanol in *n*-hexane.

After evaporation under reduced pressure the extracts were dissolved in a suitable volume of *n*-hexane and, with the spectrophotometer, the concentration was read for vitamin A at 328 $m\mu$. and for carotene at 451 $m\mu$. Further partition by separation of α - from β -carotene was accomplished by adsorption on a column of magnesia followed by elution with a 1 % solution of acetone in *n*-hexane. It was assumed that $E_{1\text{ cm.}}^{1\%}$ 451 $m\mu$. in *n*-hexane for the mixed carotenoids was 2400. Actually, however, the constant is 2500 for β -carotene and 2200 for α -carotene. $E_{1\text{ cm.}}^{1\%}$ for a sample of β -carotene supplied by British Drug Houses Ltd. was found to be 2415 in the Unicam spectrophotometer used throughout the present experiments.

All the readings for vitamin A were corrected for irrelevant absorption by the three-point method of Morton & Stubbs (1946), and expressed as international units by use of the conversion factor of 1900, attaching to the international unit and standard substance set up for vitamin A by the World Health Organization Expert Committee on Biological Standardization (1949).

RESULTS

General composition of milk. Table 1 shows the average composition of the fifty-two samples of milk. Each figure represents the average composition of all the milk samples obtained on the same day. The concentration of protein (0.63 %) was very small on the 2nd day *post partum* but became much bigger (2.01 %) the next day and remained relatively constant thereafter. Changes of fat content were different from those of protein. The initial secretion tended to be richer in fat. The increase observed by the 28th day was probably attributable to diminution of yield, an interpretation in agreement

Table 1. *The mean content of protein, fat and solids other than fat in fifty-two samples of human milk collected from 2 to 28 days after parturition*

Days <i>post</i> <i>partum</i>	No. of samples	Protein* (g./100 ml.)	Fat* (g./100 ml.)	Solids not fat* (g./100 ml.)
2	5	0.63 $\pm$ 0.06	3.4 $\pm$ 0.15	11.24 $\pm$ 0.73
3	11	2.01 $\pm$ 0.06	2.7 $\pm$ 0.28	9.77 $\pm$ 0.01
4	10	1.83 $\pm$ 0.05	3.0 $\pm$ 0.30	9.33 $\pm$ 0.16
6	12	1.74 $\pm$ 0.06	2.5 $\pm$ 0.10	9.79 $\pm$ 0.23
8	9	1.61 $\pm$ 0.05	2.9 $\pm$ 0.20	9.19 $\pm$ 0.22
10	1	1.82	3.1	9.39
12	1	1.67	2.8	9.52
28	3	1.74 $\pm$ 0.04	3.9 $\pm$ 0.27	9.26 $\pm$ 0.08

* Value with its standard error.

with that of Kon & Mawson (1950). The percentage of non-fatty solids was at a maximum on the 2nd day, was considerably less on the 3rd day and constant thereafter.

Partition of phosphorus fractions. The total content of phosphorus and the partition of the various phosphorus fractions is shown in Table 2 for thirty-four of the samples of colostrum and transitional milk collected from the 2nd to the 12th day *post partum*. The coefficient of correlation of ester phosphorus with phosphatase is shown in Table 3 together with other coefficients of correlation to which reference will be made later.

Table 2. *The mean content of phosphatase and the partition of the fractions of phosphorus in thirty-four samples of human milk collected from 2 to 12 days after parturition*

Days <i>post</i> <i>partum</i>	No. of samples	Total phosphorus* (mg./100 ml.)	Inorganic phosphorus*† (mg./100 ml.)	Ester phosphorus*† (mg./100 ml.)	Phosphatase* (arbitrary units‡)
2	4	13.3 ± 1.03	6.4 ± 0.26 (48.1)	2.8 ± 0.52 (21.1)	52.5 ± 3.7
4	10	15.9 ± 1.37	4.5 ± 0.45 (28.3)	5.3 ± 0.54 (33.3)	29.0 ± 9.8
6	10	21.7 ± 2.14	7.9 ± 0.59 (36.4)	5.6 ± 0.90 (25.8)	35.7 ± 4.2
8	8	18.6 ± 1.60	6.5 ± 0.62 (34.9)	5.4 ± 0.60 (29.0)	35.9 ± 3.9
10	1	15.7	5.3 (33.8)	6.0 (38.2)	32.2
12	1	20.4	8.0 (39.2)	6.8 (33.3)	29.5

* Value with its standard error.

† Figures in parentheses give this phosphorus expressed as a percentage of the total.

‡ Figures represent the number of mg. phenol liberated per 100 ml. milk (Kay & Graham, 1935).

Table 3. *Correlation between phosphatase and various other constituents of human milk*

Constituent	Correlation coefficient	No. of paired observations	Probability
Ester phosphorus	-0.8791	29	<0.001
Ester phosphorus (as percentage of total phosphorus)	-0.8542	29	<0.001
Inorganic phosphorus (as percentage of total phosphorus)	+0.8078	29	<0.001
Lipid phosphorus	-0.5526	16	<0.05 > 0.02
Lipid phosphorus (as percentage of total phosphorus)	-0.5171	16	<0.05 > 0.02
Free vitamin B ₁	+0.7306	24	<0.001
Free vitamin B ₁ (as percentage of total vitamin B ₁)	+0.9056	24	<0.001
Phosphorylated vitamin B ₁ (as percentage of total vitamin B ₁)	-0.9053	24	<0.001

Table 2, in which average figures are presented, and Table 3 show that the amount of ester phosphorus tended to be greater when that of phosphatase was smaller and vice versa. This correlation had been noted before in the lactating cow (Chanda & Owen, 1949) and was one of the reasons for undertaking the present researches. The relationship between phosphatase and ester phosphorus is shown in Fig. 2, in which the curve was fitted by the method of least squares. The negative correlation between ester phosphorus and phosphatase is closer than would be thought from an inspection of the standard errors shown in Table 2 because, by averaging the samples for the same day the fact has been hidden that the correlation holds also within days.

The amount of lipid phosphorus (Tables 3 and 4) also was negatively correlated with

that of phosphatase, but the amount of inorganic phosphate (Tables 2 and 3; Fig. 3) was positively correlated with the amount of phosphatase.

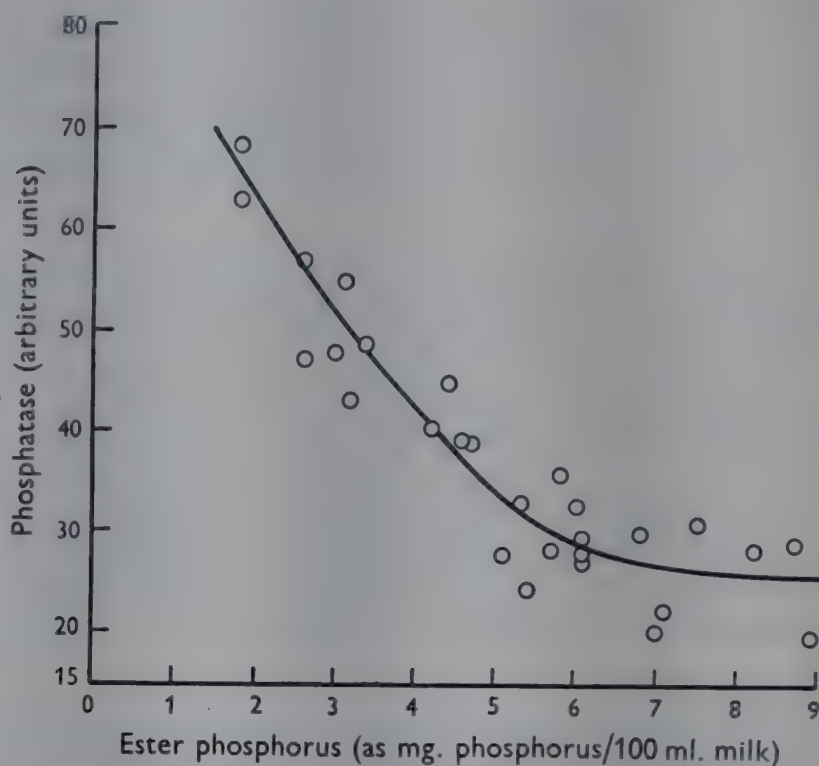


Fig. 2. Correlation between ester phosphorus and phosphatase in human milk.

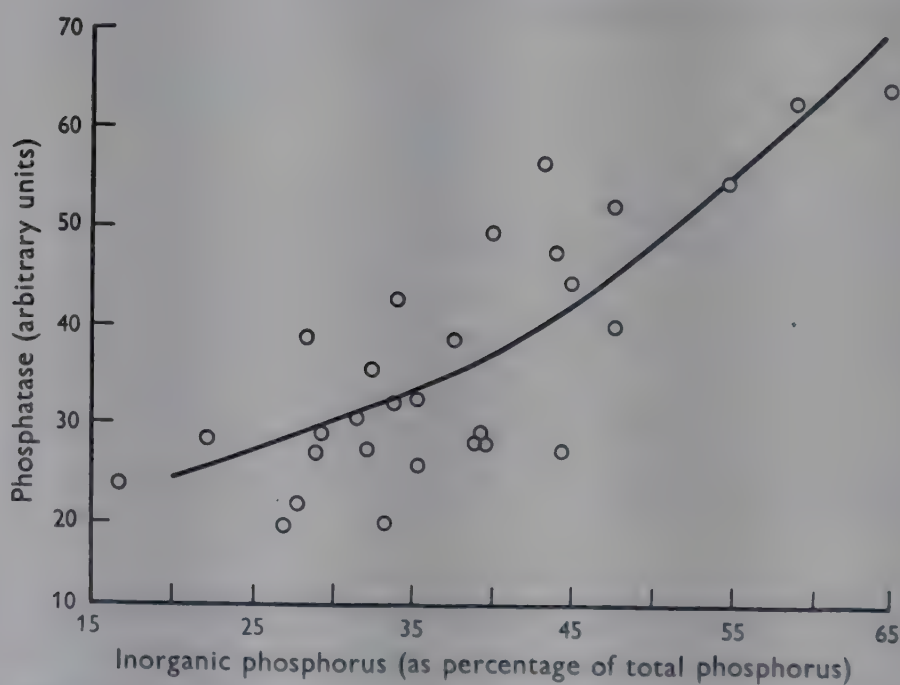


Fig. 3. Correlation between inorganic phosphorus (expressed as a percentage of the total phosphorus) and phosphatase in human milk.

Vitamin B₁ partition. The total amount of vitamin B₁ was determined in twenty-four samples; it was small at first and increased to three times its initial value after about 8 or 10 days (Table 5). Thereafter the rate of increase was slower. The ratio of phosphorylated to free vitamin B₁ increased during the 1st week *post partum* and declined thereafter. There was a large negative correlation between the amount of phosphatase

Table 4. *The amount of phosphatase and of lipid phosphorus in sixteen individual samples of human milk collected from 2 to 12 days after parturition*

Days post partum	Total phosphorus (mg./100 ml.)	Lipid phosphorus		Phosphatase (arbitrary units*)
		mg./100 ml.	As percentage of total phosphorus	
2	12.8	2.5	19.5	45.6
	11.6	1.5	12.9	62.7
4	14.4	2.8	19.4	25.9
	13.2	2.2	16.7	28.6
	18.4	4.1	22.3	27.4
	19.8	4.9	24.7	22.0
6	17.0	3.5	20.6	32.4
	16.5	3.2	19.4	56.4
	25.0	5.4	21.6	28.2
	15.0	3.1	20.7	40.0
8	14.9	2.6	17.4	44.4
	18.2	3.6	19.8	35.4
	16.2	3.8	23.5	27.0
	21.3	4.0	18.7	38.6
10	15.7	2.3	14.6	32.2
12	20.4	3.2	15.7	29.5

* Figures represent the number of mg. phenol liberated per 100 ml. milk (Kay & Graham, 1935).

Table 5. *The content of total and free vitamin B₁ in relation to the phosphatase content in twenty-four individual samples of human milk collected from 2 to 28 days after parturition*

Days post partum	Vitamin B ₁ (μg./100 ml. fat-free milk)		Phosphatase (arbitrary units*)
	Total	Free	
2	4.64	1.21	52.2
	5.91	1.49	49.5
	5.27	1.67	62.7
3	5.74	0.86	35.4
4	7.82	1.25	28.6
	6.95	0.79	22.0
	9.17	1.29	34.0
	7.52	1.26	47.6
	9.45	0.98	17.4
6	14.93	1.97	27.6
	10.73	1.68	23.6
	12.67	1.92	40.0
	11.28	1.41	32.4
	10.42	2.48	56.4
8	12.42	1.45	27.0
	9.94	1.34	35.4
	13.87	1.75	38.8
	14.56	2.98	54.5
	10.17	1.97	44.4
10	12.94	1.79	32.2
12	13.48	1.68	29.5
28	15.98	7.14	67.4
	13.77	4.28	58.9
	13.14	6.28	75.5

* Figures represent the number of mg. phenol liberated per 100 ml. milk (Kay & Graham, 1935).

and of phosphorylated vitamin B₁ expressed as a percentage of the total amount of vitamin B₁ (Table 3); Fig. 4 shows the relationship graphically. Between the amount of phosphatase and of free vitamin B₁, expressed as a percentage of the total vitamin B₁, there was an equally large positive correlation.

Nicotinic acid. Nicotinic acid was estimated only in twelve samples, by Dr M. L. McNaught and Dr C. Higginbottom. The results are shown in Table 6, and demonstrate that the nicotinic acid content continued to increase up to the 4th week of lactation, after which no further tests were made.

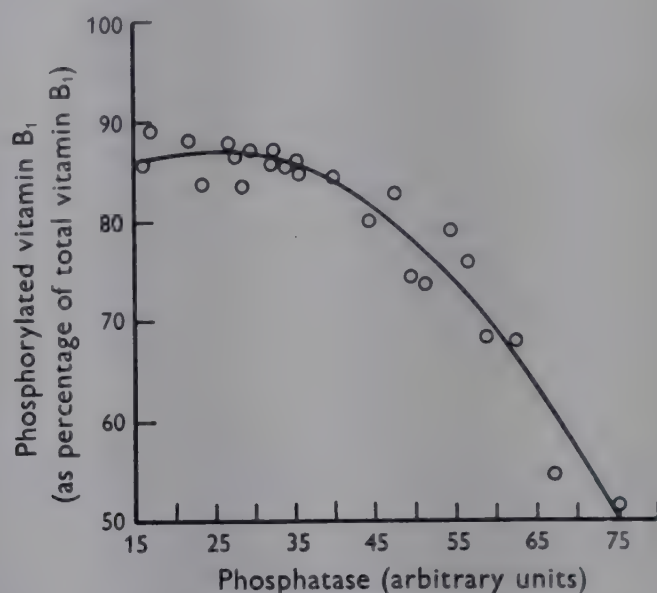


Fig. 4. Correlation between phosphorylated vitamin B₁ (expressed as a percentage of the total vitamin B₁) and phosphatase in human milk.

Table 6. *The content of nicotinic acid in twelve individual samples of human milk, collected from 2 to 28 days after parturition*

Days post partum	Nicotinic acid ($\mu\text{g.}/100\text{ ml.}$)	Days post partum	Nicotinic acid ($\mu\text{g.}/100\text{ ml.}$)
3	42	7	58
3	56	8	101
3	39	8	61
4	86	28	103
4	81	28	180
7	97	28	226

Carotenoids and vitamin A. Carotenoids and vitamin A were estimated in twenty-six samples of milk; the results are recorded in Tables 7 and 8. Chromatography showed that the fat from human milk contained xanthophylls, α - and β -carotenes and lycopene, of which the last three were measured spectrophotometrically. The presence of lycopene in human milk was reported by Thompson, Kon & Mawson (1942). That the red carotenoid was lycopene in this instance also was demonstrated by comparing its properties with those of lycopene prepared from tomato. The pigments from the two sources showed only a single band when mixed and chromatographed. Their absorption spectra, in *n*-hexane solution, between 300 and 600 $m\mu$. were compared and are shown graphically in Fig. 1. The two curves are of the same shape and both have maxima at

505, 475 and 448 mμ., in accordance with the findings of Morton (1942), that the absorption maxima for lycopene in light petroleum are at 506, 474 and 444 mμ.

Eight samples of milk were examined for α- and β-carotene separately, and both were identified by their spectral absorption and their chromatographic properties. Pure α-carotene and the mixtures of α- and β-carotenes obtained from carrots and from

Table 7. *Distribution of carotenoids and of the alcohol and ester fractions of vitamin A in twenty-six individual samples of human milk, collected from 3 to 28 days after parturition*

Days post partum	Carotenoids*			Vitamin A			Fat (%)	Yield of milk (ml. daily)
	Total (μg./100 ml.)	α- and β-carotene (% of total)	Lycopene (% of total)	Ester (i.u./100 ml.)	Alcohol (i.u./100 ml.)	Total (i.u./g. fat)		
3	120.5	24.4	50.1	254.6	27.4	156.7	1.8	130
	145.3	25.9	49.9	600.4	49.2	162.4	4.0	215
	137.2	28.9	48.3	359.1	45.1	202.1	2.0	117
	118.1	28.7	46.0	318.4	32.0	206.1	1.7	263
	109.7	38.7	31.2	522.1	57.8	148.7	3.9	363
	114.5	27.3	40.5	419.3	29.4	187.0	2.4	295
	99.4	22.3	52.4	304.2	19.9	190.6	1.7	150
	112.3	24.7	43.8	450.3	26.6	153.8	3.1	135
	125.6	24.8	40.0	547.2	39.3	162.9	3.6	205
	87.4	27.9	44.1	388.1	21.9	186.4	2.2	668
4	76.4	28.4	40.7	311.7	19.3	132.4	2.5	515
	112.3	25.9	44.3	450.0	37.2	101.5	4.8	187
	107.6	23.8	48.9	237.0	20.7	88.9	2.9	965
5	96.7	28.3	45.9	215.5	36.1	125.8	2.0	100
	87.5	22.3	47.3	310.3	24.0	88.0	3.8	365
	115.4	28.2	42.7	264.3	42.4	95.8	3.2	445
6	58.3	28.1	34.7	185.5	20.9	108.6	1.9	120
	67.2	25.4	34.8	318.4	25.4	86.0	4.0	235
	47.6	26.3	36.8	294.1	16.5	110.9	2.8	292
7	45.4	34.1	34.1	221.1	17.2	68.1	3.5	445
	39.2	28.6	40.6	264.0	14.9	84.5	3.3	500
8	41.4	32.1	17.9	195.7	17.5	71.1	3.0	535
	36.7	28.3	33.2	272.8	15.5	60.1	4.8	580
						63.2	4.2	540
28	37.6	33.0	27.4	247.0	18.5	56.2	4.2	530
	33.8	29.0	39.7	223.4	12.8	66.4	3.4	575
	48.4	30.2	40.1	207.8	17.9			

* The total carotenoids include, besides α- and β-carotene and lycopene, other carotenoids without vitamin A potency, absorbing at 451 mμ., of which the chief is xanthophyll (lutein).

red palm oil were used for comparison to confirm the identity of the milk pigments. Quantitative partition of carotene into α- and β-fractions is recorded in Table 8. The concentration of both carotenes decreased in the milk as lactation progressed, the fall in the α-fraction being the greater.

It is clear from Table 7 that the content of biologically active carotenoids in human milk was small relative to the concentration of preformed vitamin A, and that the infant must rely little if at all on them as a source of vitamin A potency. The result is in agreement with that of Kon & Mawson (1950). Most of the vitamin A, as is seen from Table 7, was in the form of the ester.

Table 8. *Content of α - and β -carotene and total carotene in eight individual samples of human milk, collected from 3 to 28 days after parturition*

Days post partum	Carotene content			Ratio β -carotene: α -carotene
	Total ($\mu\text{g./100 ml.}$)	β -carotene ($\mu\text{g./100 ml.}$)	α -carotene ($\mu\text{g./100 ml.}$)	
3	29.4	17.7	10.4	1.70
3	42.5	25.8	15.9	1.62
3	31.1	17.8	12.5	1.42
4	25.6	19.9	10.9	1.83
5	19.5	13.2	6.1	2.16
6	16.4	11.4	4.5	2.53
7	11.2	6.9	3.7	1.87
28	9.7	6.5	2.1	3.10

DISCUSSION

Protein. Our observations showed that human lactation differs from that of the cow in the behaviour of the protein content of the secretion. The samples of human colostrum studied had a smaller concentration of protein than those of the later milk, whereas cow's colostrum is richer in protein than the later milk. The concentration of protein increased as lactation proceeded, whereas in the cow the colostrum, initially rich in protein, gives way to milk of smaller protein content. The differences may perhaps be related to the richness of cow's colostrum in globulin with its accompanying content of antibodies. In the human being such antibodies are chiefly acquired *in utero*, and the neonatal requirement for globulin is consequently less. The calf grows more rapidly than the human infant, and therefore needs a liberal intake of calcium phosphate for bone growth, which in turn necessitates a larger content of casein in the milk in order to prevent the precipitation of calcium phosphate.

Nitrogen and phosphorus. According to Owen (1948), the ratio of nitrogen to phosphorus in cow's milk shows variations attributable to the activity of the thyroid gland, because thyroxine increases the phosphorus content of the milk without much affecting the concentration of nitrogen or of calcium. In cows the average percentage concentration of nitrogen is 0.6 and of phosphorus 0.1, giving a ratio of N:P of 6:1. The present figures give percentages for human milk of 0.300 and 0.015, and a ratio of N:P of 20:1.

Observations now being prepared for publication show that in the cow the N:P ratio was reduced to about 5 by treatment with thyroxine or increased to about 7 by treatment with thiouracil. Administration of thyroxine increases total phosphorus in cow's milk by from 15 to 20 % (Owen, 1948; Chanda & Owen, 1949) and thiouracil decreases it from 10 to 15 % (Chanda & Owen, 1949).

Phosphatase. In the present experiments the amount of phosphatase in human colostrum and milk varied in a manner similar to that found in other species. Chanda & Owen (1949) found a significant negative coefficient of correlation between the amounts of ester phosphorus and of phosphatase in cow's milk. The coefficient increased on treatment with thyroxine or thiouracil. Further studies of colostrum and late milk of cows showed that this correlation was true throughout lactation, in spite of the large variations in the amount of phosphatase demonstrated by Folley & Kay (1936-7). By

analogy, therefore, between the bovine and human species it would be expected that the much smaller phosphatase titre of human milk would be associated with a much greater proportion of ester phosphorus. This was indeed found to be so in the present investigations, for human milk had from 21 to 38 % of the phosphorus as ester, whereas in cow's milk the percentage was only 10. Later observations, now being prepared for the press, show that in the concentrations of phosphatase and of ester phosphorus, the goat resembles the human being and not the cow. Basu & Mukherjee (1943), however, found that the amounts of ester phosphorus in goat's milk were smaller, and similar to those in cow's milk; their results are thus at variance with the present observations. They did not, however, appreciate the necessity for comparing milk at similar stages of lactation.

Some correlations of milk constituents are recorded in Table 3, which shows that the amounts of lipid phosphorus and of phosphorylated vitamin B₁ also were negatively correlated with the amount of phosphatase, but there was a positive correlation between the amounts of inorganic phosphate and of phosphatase. These correlations are consistent with the hypothesis that ester phosphorus is the intermediary in metabolism between inorganic phosphate and lipid and casein phosphorus. The results of Chanda & Owen (1949), already cited, also are consistent with that hypothesis. The intermediary ester phosphorus may be supposed to be synthesized in the mammary epithelium by phosphatase from inorganic phosphate. The correlation between the amount of phosphatase and the percentage of phosphorylated vitamin B₁ was -0.905 (Table 3). The closeness of the correlation is shown by Fig. 4. As much as 90 % of the vitamin B₁ was present in human milk in the phosphorylated form, a value which may be compared with 30 % found by Neuweiler (1941) who, however, did not record the days *post partum* on which his samples were collected. The present experiments gave the percentage as from 52 to 69 even on the 28th day *post partum*. The inverse correlation between the amount of phosphatase and the ratio of phosphorylated to total vitamin B₁ has been previously noted in cow's and goat's milk by Houston *et al.* (1940), in cow's milk by Chanda *et al.* (1949) and in sow's milk by Braude, Coates, Henry, Kon, Rowland, Thompson & Walker (1947). Bartlett, Rowland & Thompson (1949) observed a decreased ratio of free to total vitamin B₁ in the milk of cows ingesting thyroxine or iodinated protein; they did not record the amount of phosphatase but clearly stated the trend. A noteworthy feature of the experiments of Chanda & Owen (1949) was the use of thiouracil to produce hypothyroid animals. In these animals the ratio of the amounts of phosphorylated to total vitamin B₁, the percentage of phosphoric esters, and the percentage of phospholipin declined as the amount of phosphatase increased, so that the large negative correlations between the amounts of phosphatase and of other milk constituents still persisted. The persistent interdependence of the amounts of phosphorylated compounds and phosphatase is probably related to the power of thyroxine to increase the concentration of enzymes in the body. Such an increase would necessitate an increase of the body's phosphorylated coenzymes which are so important in energy transfers.

Vitamin B₁. The total concentration of vitamin B₁ in the milk (Table 5) increased steadily as lactation progressed, becoming steady after the 8th day, and an inspection

of the milk yields in Table 7 shows that the output of vitamin B₁ also must have increased considerably as lactation progressed. The amounts of vitamin B₁ in the milk were larger than those reported by Kendall (1942), and from the United States by Roderuck, Williams & Macy (1945). The observations of Kon & Mawson (1950) suggest that large variations in the vitamin B₁ content of the milk are to be expected according to the degree of extraction of the bread flour in the mothers' diets and whether it is fortified with vitamin B₁ or not. That the intake of vitamin B₁ affects the amount in the milk is generally agreed (Slater & Rial, 1942; Escudero & de Esquef, 1944; Roderuck *et al.* 1945; Clements, 1949; Kon & Mawson, 1950).

Carotenoids and vitamin A. The occurrence of lycopene in human milk (Thompson *et al.* 1942) and in cow's milk has previously been observed and it is known to be of dietary origin (Thompson, Ganguly, Mawson & Kon, 1949). We do not know to what extent tomatoes or swede turnips contributed to the occurrence of lycopene in the present samples. The occurrence of β -carotene is attributable to consumption of any greenstuff, but its occurrence with α -carotene is probably due to the eating of carrots. The great variation between samples in the content of vitamin A is perhaps due to failure on the part of some of the mothers to take the vitamin capsules supplied by the welfare centres and hospitals.

SUMMARY

1. Samples of normal milk from mothers delivered in the Aberdeen Maternity Hospital were collected at intervals up to 28 days after parturition, and were analysed for certain constituents, with the following results.
2. The percentage of protein was very small, 0.63, on the 2nd day *post partum*, but rose to 2.01 the next day and remained at about that level thereafter.
3. The percentage of fat ranged from 2.5 to 3.9 and was much more variable than that of protein.
4. The percentage of solids-not-fat varied from 9 to 10 with an initial figure of about 11.2.
5. The amounts of inorganic, ester and lipid phosphorus were closely correlated with the phosphatase content of the milk, that of inorganic phosphorus positively, and of ester and of lipid phosphorus negatively. The amount of phosphatase in the milk was large at first, falling as lactation progressed.
6. The N:P ratio was 20 in human milk, compared with only 6 in cow's milk.
7. The amount of nicotinic acid increased from 42 μ g./100 ml. on the 3rd day *post partum* to 226 μ g./100 ml. on the 28th day.
8. Phosphorylated vitamin B₁ behaved like ester phosphorus in its relationship to phosphatase, the negative correlation between the amounts of the two being close.
9. The amount of total vitamin B₁ increased steadily as lactation progressed. Comparison of the values obtained with those published by others served to strengthen the general belief that the amount of vitamin B₁ in the milk is readily affected by dietary intake.
10. On the basis of the present analyses an infant's total intake of vitamin A could vary very widely.

11. The vitamin A activity of the milk was mainly due to vitamin A itself; the contribution from carotenes was very small. Of the vitamin A more than 90 % was in the ester form.
12. Two-thirds to four-fifths of the activity of the carotenes was due to β -carotene and the remainder to α -carotene.
13. Lycopene, which is not a precursor of vitamin A, formed one-quarter to one-half of the total carotenoids. Inactive xanthophylls were present also, but no attempt was made to estimate them quantitatively.
14. The vitamin A concentration in the milk fat was larger at first than later in lactation.
15. The results for human milk are compared with those obtained recently for cow's milk.

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PROCEEDINGS OF THE NUTRITION SOCIETY

SIXTY-FOURTH SCIENTIFIC MEETING
LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

20 JANUARY 1951

THE COMPARATIVE MERITS OF ANIMAL AND VEGETABLE FOODS IN NUTRITION

*Chairman: PROFESSOR R. C. GARRY, Institute of Physiology,
University of Glasgow*

Chairman's Opening Remarks

By R. C. GARRY, Institute of Physiology, University of Glasgow

Controversy concerning the relative nutritive merits of protein from plant and animal sources is of long standing. Our conception of the total quantity of protein required has also swung wildly from one extreme to the other. This scientific problem, intrinsically difficult in itself, has been, and still is, emotionally bedevilled by prejudice and sentiment.

Recent advances in biochemistry have given us a better appreciation of the ultimate composition of proteins from different sources, and have helped to explain and foretell the 'biological values' of different proteins. Nevertheless, we must not forget that we do not eat proteins as such, we eat food containing protein. And evidence is accumulating that the value of the protein may depend to some extent on the vehicle in which it is presented. The time is propitious for stocktaking, for a review of the past, for a forecast of the future. This is the purpose of our conference to-day.

Biochemistry of Animal and Vegetable Proteins

By G. R. TRISTRAM, University of St Andrews

Text not received for publication.

The Relative Nutritional Values of Animal and Vegetable Proteins for Animals

By K. J. CARPENTER, Rowett Research Institute, Bucksburn, Aberdeenshire

The classical method for the nutritional evaluation of the protein complex in individual foods or feeding-stuffs is to feed them, at a level of 10% protein, as the sole protein source in the otherwise adequate diet of young, growing rats. The material is then rated either by its digestibility and biological value (the proportion of the absorbed

nitrogen which escapes excretion in the urine) or by the protein-efficiency ratio (the gain in weight of the rat per g. protein eaten). From an examination of published data for thirty-eight materials Block & Mitchell (1946-7) found a very high degree of correlation ($r = +0.84$) between the protein-efficiency ratio and the net protein utilization (digestibility $\times$ biological value).

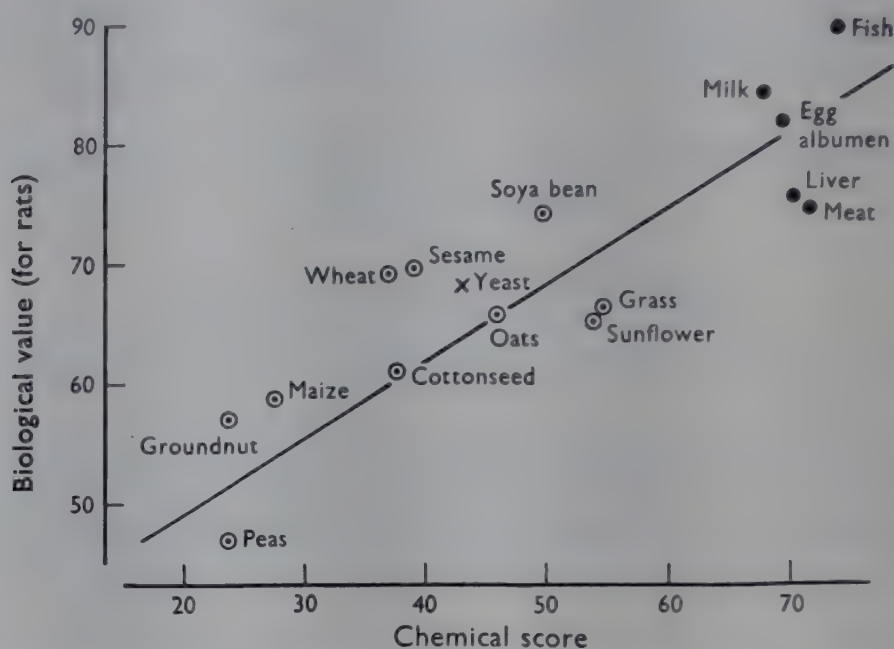


Fig. 1. Correlation diagram of the biological value and chemical score of sixteen animal (●) and vegetable (○) materials. (Data from Mitchell & Block, 1946; Bartlett, Henry, Kon, Osborne, Thompson & Tinsley, 1938; Harrison, Anderson & Pottinger, 1935.)

Figures of fair reliability are available for the essential amino-acid content of most common feeding-stuffs, and for these Block & Mitchell have calculated a chemical score in terms of whole-egg protein, which is almost wholly utilized by the young rat. This is obtained by calculating the content of each essential amino-acid in the protein ($N \times 6.25$) of a material as a percentage of the concentration of the same amino-acid in whole-egg protein. As the limiting amino-acid is held to determine the value of the whole protein, the lowest percentage obtained is used as the chemical score.

In Fig. 1 some of the available data (mostly from Mitchell & Block, 1946) for the chemical score and biological value of materials of interest in animal nutrition are set out in diagrammatic form. Again there is a high degree of correlation between the two methods of evaluation. The data show the general superiority of the animal materials over those of vegetable origin. Only soya-bean meal, toasted to destroy the trypsin inhibitor it contains, is within the range of the animal proteins.

These figures for biological values would be invalidated if it were shown that the animal materials alone carried with them vitamins which affected the utilization of protein, and in which the rat was deficient.

Vitamin B₁₂-deficient rats have an increased requirement for methionine (Schaefer, Salmon & Strength, 1949) and the work of Bosshardt, Ayres, Ydse & Barnes (1946) suggests that utilization of dietary protein will be impaired. This vitamin is found in animal materials, but is absent from unfermented vegetables, as also from the basal diet

used in the trials referred to. Nevertheless, the rats will almost certainly have carried sufficient reserves of vitamin B₁₂ from their suckling period to prevent a deficiency in the short experimental period (Zucker & Zucker, 1948). This is confirmed by the biological values for the vegetable, as compared with the animal, feeds being at least as great as would be expected from the relative chemical scores of the two classes.

These rat experiments represent necessarily a great simplification of the practical problem of making up balanced rations at low cost. There remain the possibilities of less exacting requirements in later life (when a much greater aggregate of feed is consumed), mutual supplementation between vegetable proteins, and species differences.

Cattle and sheep

The ruminants, with the exception of the period when they are suckling (Blaxter & Wood, 1950), have an alimentary microflora encouraged by the dynamics of the digestive system to attack the feed for a considerable time. The micro-organisms have wide powers of synthesis. When urea is given as the sole source of nitrogen, all ten of the essential amino-acids are found in the rumen in approximately the same quantities as after feeding a good-quality protein (Thomas, Loosli, Ferris, Williams & Maynard, 1949).

It is not surprising, therefore, that for ruminants the proteins in the common feeding-stuffs, whether animal or vegetable, are generally similar in biological value (McNaught & Smith, 1947). Moir & Stewart (1947) showed that legume seeds low in the sulphur amino-acids were of low value in promoting heavy wool growth, but a requirement by sheep for dietary cystine and methionine has not yet been proved.

The major feed of both cattle and sheep is fresh herbage, which should meet their maintenance requirements for protein, and even sustain a moderate level of production.

Pigs and poultry

Pigs and poultry, both monogastric species, cannot tolerate the high level of fibre in a ration composed mainly of grass, and in practice the cereal grains and offals form the main source of energy in their rations. For poultry of all ages and for pigs (except during fattening), a mixture of cereals is deficient in protein. The practical problem is therefore to assess the relative values of animal and vegetable proteins as supplements to cereals for these two species.

So far, individual amino-acid requirements have been worked out only for chicks. Calculations suggest that of all the essential amino-acids only lysine and the sulphur-containing amino-acids, cystine and methionine, will be limiting factors in practical rations, and their concentration in sixteen feeding-stuffs (De Man, 1949) is shown diagrammatically in Fig. 2.

One criticism of experiments with supplementary proteins is that the results may apply only for the particular basal mixture used. However, the similar composition of the main cereals suggests that supplementary values should not differ greatly with the cereal mixture. This was confirmed when maize and wheat were tested separately with a series of fish meals and meat meals (March, Stupich & Biely, 1949).

The approximate percentages of lysine and cystine + methionine required in the protein of a chick ration containing 20% protein have been determined (Almquist, 1947) and are shown in Fig. 2. By analysis, the cereals should be deficient in lysine and border-line for the sulphur amino-acids, and this was confirmed for a wheat-protein preparation (Jeppesen & Grau, 1948).

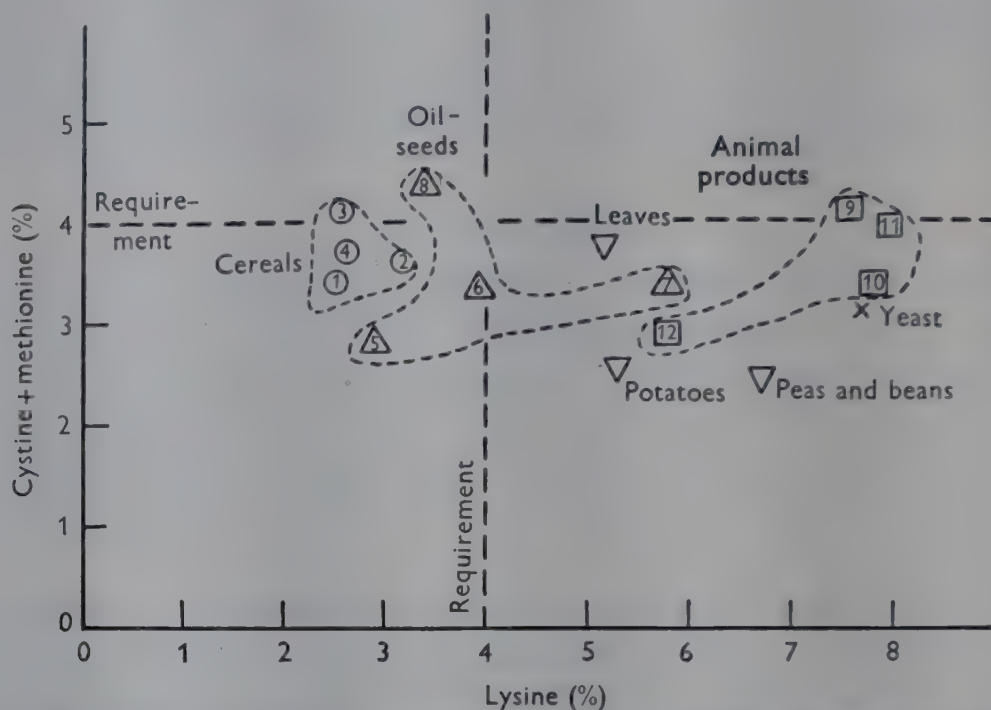


Fig. 2. Lysine and 'cystine + methionine' in the crude protein of sixteen feeding-stuffs, and the requirement of these amino-acids by the chick in an 'ideal' protein fed at 20% level. In each case the requirement is 0.8% of the total ration or, as shown here, 4.0% of the protein. (Data from Almquist 1947; De Man, 1949; and Grau & Kamei, 1950.) 1. Barley. 2. Oats. 3. Maize. 4. Wheat. 5. Groundnut. 6. Cottonseed. 7. Soya-bean. 8. Sunflower. 9. Fish meal. 10. Blood meal. 11. Skim milk. 12. Meat meal.

The common animal feeding-stuffs are generally higher in lysine than the vegetable feeds, and so appear better able to supplement the cereals. Soya-bean meal again appears to be outstanding among the vegetable proteins. Meat meal is inferior to the other animal feeds by analysis, and a large proportion of the lysine may also be unavailable to the chick (March, Biely & Young, 1950). The lysine of groundnut meal, though present at a low level, is mostly available (Carpenter & Ellinger, 1951).

There are some differences between the methionine content of feeds as estimated by chemical and microbiological methods (De Man, 1949), but it is clear that though some of both classes of supplements are deficient in the sulphur-containing amino-acids, no protein has a compensating excess.

Heiman, Carver & Cook (1939) have suggested a standard method of evaluating supplementary proteins for chicks, using a basal 8% protein ration of mixed cereals and vitamin concentrates. The supplements are added, for different groups, to give 3% additional protein. Their gross protein value (G.P.V.) is the extra growth obtained (in 2 weeks) divided by the supplementary protein eaten. The values are expressed as a percentage of that obtained for casein.

Table 1 shows the results obtained for a series of feeding-stuffs with the original

method (Robertson, Carver & Cook, 1940), and with the crude-fibre level kept constant (Carpenter, Duckworth & Ellinger, 1951). The results for animal materials, and for extracted oilseeds are in good agreement with the expectations based on their amino-acid composition. The low value for cottonseed meal may be attributed to the presence of toxic material in the sample (Ingram, Cravens & Elvehjem, 1950).

Table 1. *Gross value* of protein supplements for chicks (casein = 100)*

(The figures are the mean values obtained with each feeding-stuff)

Supplement	U.S.† results	U.K.‡ results
Animal products:		
Casein	100	100
Herring meal	101	95
White fish meal	—	89
Dried skim milk	90	—
Meat meal	55	—
Oilseeds:		
Soya-bean meal	76	—
Groundnut meal	—	50
Cottonseed meal	25	—
Coconut meal	22	—
Herbage:		
Lucerne meal	37	27
Lucerne meal and 0.15 % cholesterol	—	70
Grass meal	—	55
Grass meal and 0.15 % cholesterol	—	58
Red clover meal	—	46

* For definition see p. 246.

† Robertson *et al.* (1940).

‡ Carpenter *et al.* (1951).

The first value obtained for lucerne meal was low, considering the promising analyses obtained for leafy materials. However, Peterson (1950) showed that lucerne contains a 'saponin-like' growth-depressant inactivated by the addition of cholesterol, and the G.P.V. for lucerne was greatly increased by adding 0.15 % cholesterol to the ration. Ordinary grass meal appeared not to contain this growth-depressant to any significant extent. The values for the leafy materials tested are still lower than for soya-bean meal, though amino-acid analyses suggest that they should be of approximately equal value. The analyses may be wrong, or alternatively the leaf proteins may be less digestible. Of the sixteen materials for which data from rat experiments are given in Fig. 1 above, all had a digestibility greater than 90 %, with the exception of grass meal for which the figure was 67 %.

If it is accepted that vegetable protein supplements are generally inferior to the animal ones, the problem is to determine how far the inferiority can be made up by giving the supplementary protein at higher levels.

Fig. 3, based on the results of three comparable chick trials lasting 4–6 weeks, shows the findings with herring meal and groundnut meal as supplementary proteins. The growth rates converge as the level of supplementation increases. The proportion, however, in which the two supplements have to be fed in order to produce any given

growth rate is constant (in this instance 2 : 1). This would be expected if the requirement for the individual amino-acids remained constant within the range of protein levels used.

It has been shown by Grau & Kamei (1950) that the individual requirements for lysine and methionine increase when the protein level of the chick ration is raised to 30 or 40%. With a sufficiently unbalanced protein, additional supplementation should then make things worse rather than better. With groundnut meal, one of the poorer protein sources in common use, this does not occur, and the leeway can be made up by increasing the level (Fig. 3).

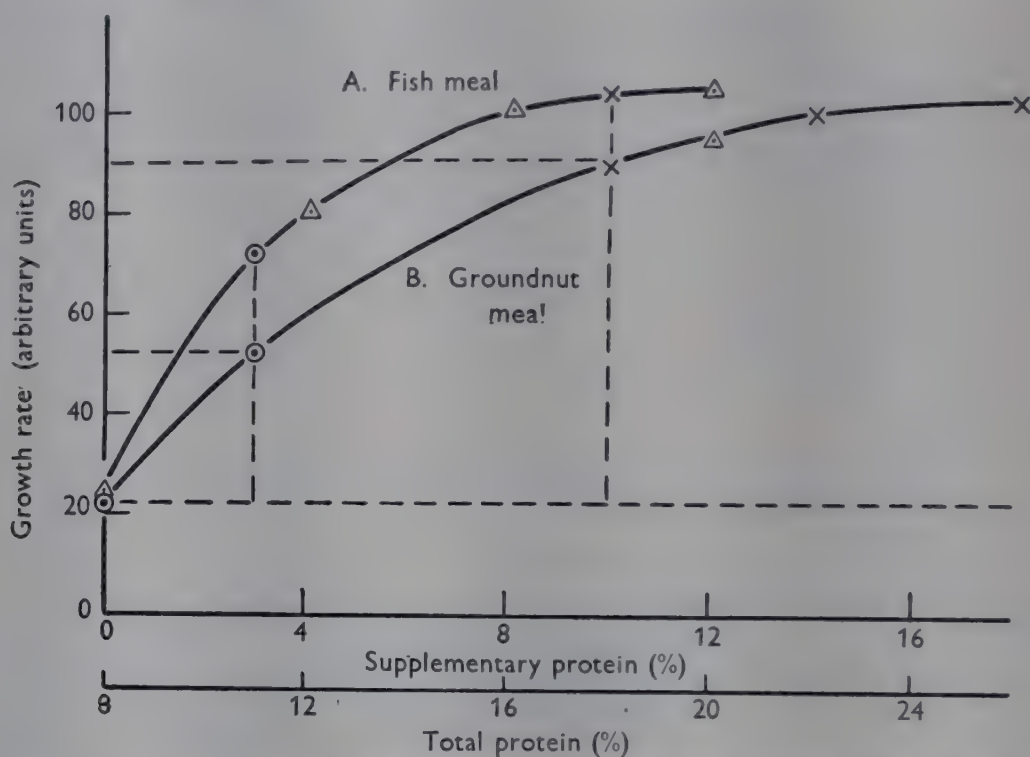


Fig. 3. Growth of chicks in the first 4-6 weeks of life according to protein level, and nature of protein supplement. Combined data from three experiments (Carpenter *et al.* 1951). Δ = Exp. 1; $\odot$ = Exp. 2; $\times$ = Exp. 3.

In practice, high initial growth rates are not an end in themselves, and we have found that a groundnut ration of normal protein level will finally produce a healthy bird at point of lay with the same feed-conversion efficiency as will a fish-meal ration giving a higher growth rate for the first few weeks (cf. Halnan, 1948).

In laying trials, with rations containing a total of 14-15% protein, fish meal has been replaced, without any significant differences resulting, by either soya-bean meal (Forrest, Biely & March, 1950), sesame meal (Hale & Bolton, 1948) or groundnut meal with palm-kernel meal (Temperton & Dudley, 1939-40). This contrast with the chick results could be due to a greater ability of the older animal to synthesize some of the essential amino-acids. A simpler explanation may be that the fish meal in the rations is in excess and could be reduced without effect, but that the level of the vegetable proteins could not be reduced.

General conclusions

Animal protein feeding-stuffs such as fish meals and dried skim milk are rich sources of vitamins, important for non-ruminant livestock. The recent introduction of

condensed fish solubles, 'animal protein factor' concentrates from the antibiotics industry and synthetic riboflavin as alternative sources of these vitamins will provide a greater field for the use of otherwise suitable vegetable proteins which are not also potent sources of these factors. Research has shown that they are generally inferior to animal proteins, but that this may be made up for by feeding them at higher levels. Whether such a change is economically worthwhile will depend upon the cost of any extra vitamin supplements needed, as well as on the relative cost of the protein supplements themselves.

The use of vegetable proteins may be particularly important in the colonial development areas where fish meal and milk products are not normally available for pig and poultry feeding. Unfortunately, ordinary grass and leaf meals are high in fibre, and cottonseed meal contains a growth depressant. These limit their usefulness at present, but new processing methods may be worked out to overcome these difficulties.

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Conversion Factors for Vegetable and Animal Foods for Human Consumption

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AGRICULTURAL RESEARCH COUNCIL)

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To translate animal and vegetable foods, meat, milk, eggs, cereals and vegetables, into terms of human consumption and then to discuss their adequacy in meeting the nutritional demands of man, necessarily involves decisions on common denominators. During translocation of crop or animal from the farm to the consumer many inevitable losses occur and, even in the hands of the consumer, further considerable, culinary, waste takes place. The first problem is, therefore, to decide on the unit to be used to assess this wastage. It could be a monetary one—the amount of food purchased for a given amount of money—or a unit of human endeavour—the amount of food produced per unit of man-power. Production per acre of agricultural land, however, is probably the most generally acceptable unit to use, and this I have chosen. The second problem is concerned with evaluation in terms of human needs. So far we have been concerned solely with protein supplied from vegetable and animal products, in some instances partly purified. We must also think in terms of dietary energy, essential vitamins, minerals and possibly as yet unidentified nutrients.

I have, therefore, considered requirements not only for protein but also for dietary energy, calcium, vitamin C, vitamin B₁ and vitamin A. As, however, most of the discussion on the differences between, or complementary virtues of, vegetable and animal foods revolves around their respective proteins, protein supplies from these two sources must be evaluated in terms of their ability to meet protein requirements. Biological values of single proteins have little practical value in this respect, since the biological value of the proteins of a mixture of two foods is not the arithmetic mean of the biological values of the proteins of the two foods determined separately (Mitchell, 1924). Even supplementation of the medium-quality proteins of wheat with the poor-quality proteins of gelatin results in a biological value of the mixture greater than that of the wheat proteins alone (Chick & Slack, 1945).

A solution to this difficulty is to express human requirements in terms of essential amino-acids. Requirements for amino-acids should be additive. Table 1 shows three estimates of the daily amino-acid requirement of man (Rose, 1949; Block & Bolling, 1945; Macy, 1943). It will be noted that the values given by Rose are considerably less than those given by the other authors. Rose's values, however, are double the requirement for the particular amino-acid necessary to maintain nitrogen equilibrium in young men, and as such are the only experimental values so far available. That they are approximately correct may be seen in Table 2, where the minimum requirements for particular proteins for the maintenance of nitrogen equilibrium have been expressed in terms of amino-acids. The amino-acid present in limiting concentration—lysine for wheat flour and methionine for milk proteins—is present in amounts almost

Table 1. Recommended daily requirement of amino-acids for an adult human subject

Amino-acid	Experimentally determined by Rose (1949)* (g.)	Calculated from analysis of diets	
		Block & Bolling † (1945) (g.)	Macy (1943) (g.)
Tryptophan	0.5	1.1	0.9
Phenylalanine	2.2	4.4	4.2
Lysine	1.6	5.2	4.6
Threonine	1.0	3.5	3.2
Valine	1.6	3.8	3.2
Methionine	2.2	†	†
Leucine	2.2	9.1	9.6
Isoleucine	1.4	3.3	3.1
Histidine	Not required	2.0	1.6
Cystine and methionine	—	3.8	3.7

* Requirement taken as double the mean requirement necessary to maintain nitrogen equilibrium in a young adult male.

† See cystine and methionine.

Table 2. Daily protein requirement for maintenance of nitrogen equilibrium in an adult human subject with basal metabolic rate of 1700 Cal./24 hr., expressed in terms of essential amino-acids

Amino-acid	Proteins		Mean requirement of amino-acid for nitrogen equilibrium (Rose, 1949) (g.)
	Whole milk, 29.3 g.* (g.)	Wheat flour, 50.6 g.* (g.)	
Tryptophan	0.47	0.40	0.25
Phenylalanine	1.67	2.75	1.1
Lysine	2.19	0.90†	0.8
Threonine	1.35	1.35	0.5
Valine	1.93	2.50	0.8
Methionine	1.08†	1.50	1.1
Leucine	3.31	4.50	1.1
Isoleucine	1.81	2.25	0.7

* Bricker, Mitchell & Kinsman (1945).

† Limiting amino-acid in the nutrition of man when this source of protein used.

identical with those experimentally determined by Rose. These requirements do not allow for adult growth, loss of skin debris, growth of epidermal structures and minor changes of body form (Bricker, Mitchell & Kinsman, 1945; Hrdlička, 1936). Results of balance experiments have shown that this adult growth and loss of epidermal tissues is approximately 0.98 mg. nitrogen/basal Cal. of heat produced. This effectively doubles the protein requirement for mere nitrogen equilibrium. The recommended allowances of Rose, which are arbitrarily double the minimum, appear, therefore, to be quite suitable as standards of requirement, and better than those based on analysis of diets.

In Table 3 are listed the requirements of adult man for a whole year, based on Rose's estimates of amino-acid requirement, and the (U.S.A.) National Research Council's (1943) recommended allowances. Only tryptophan, lysine and methionine have been included in the estimates of requirement, as it seems unlikely that other amino-acids would ever be short.

Table 3. *Daily and yearly requirements of an adult human subject for certain nutrients*

Nutrient	Requirement	
	Daily	Yearly
Calories	3000 Cal.*	1.1 million Cal.
Tryptophan	0.5 g.†	182 g.
Lysine	1.6 g.†	584 g.
Methionine	2.2 g.†	803 g.
Calcium	800 mg.*	292 g.
Aneurin	2.0 mg.‡	730 mg.‡
Ascorbic acid	75 mg.*	27.4 g.
Vitamin A	5000 i.u.*	1.8 million i.u.

* (U.S.A.) National Research Council (1943). Recommended daily allowances.
† Rose (1949). Recommended daily allowances.
‡ Probably too high an estimate, especially for high-fat diets.

In the conversion of animal and vegetable crops into human food many losses undoubtedly occur as, for example, in the production of sugar from sugar beet and of flour from wheat. The losses of gross calorific value of the two crops are very considerable indeed, and Table 4 shows that large amounts of by-products are retained on, or sent back to, the farm, where they can be utilized by livestock. This point will be considered later.

The computed amounts of human nutrients produced per acre of land by animal and vegetable crops, disregarding for the present this return of by-products and their utilization by animals, are shown in Table 5. As we all know, wheat, sugar beet and potatoes are primary sources of dietary energy for the population, whereas animal products from a similar acreage supply only one-tenth to one-quarter as much. An acre of good agricultural land sown to wheat could supply the calorie needs of 2.4 people for a year, but could supply sufficient lysine for only 1.8, little, if any, ascorbic acid or vitamin A activity, and, without fortification of the flour with chalk, could supply the calcium needs of only 0.3 people. Except possibly for the lack of ascorbic acid in the egg, the utilization of the calories of the animal foods is not limited by the supply of amino-acids, calcium or vitamins B₁, C or A. Yet milk could supply the lysine needs of 4.1 people, the calcium needs of 3.6 and the vitamin A needs of nearly 2.0. In other words, the total nutritive value of the main vegetable crops is limited by their shortage of vitamins and, to a much lesser extent, of essential amino-acids, whereas animal crops provide a superabundance of these nutrients but a shortage of calories. What the housewife calls vegetables, i.e. green crops of various sorts, occupy a special position. They supply considerable excesses of vitamins A and C and in addition provide large quantities of essential amino-acids. Bulkiness limits their usefulness, since they do not supply a large number of calories. An adequate human diet can be planned from an arable acreage including a green crop, but it would not necessarily be palatable. A further point is that an entirely vegetable dietary for man is not necessary from the point of view of maximal production from our land resources, since by-products have to be utilized. This entails feeding to livestock the waste products of the food industry as well as the utilization of the straw, tops and unsaleable produce left on the farm.

Table 4. Conversion of sugar beet and wheat crops into human food, direct products for human consumption from 1 acre of average farm land

Crop	Total saleable produce	By-products			Processing losses	Total yield for direct human consumption	
		Retained	Returned to agriculture from factory	Miller's offals; part of screenings		Weight	As percentage of total dry matter of whole crop
Wheat	Grain, 18 cwt.	Small corn } 1.5 cwt.	Straw, 1 ton	Tops and crowns, 7 tons	Cleaning, 2 %	Flour, 850 kg.	50 (including straw)
		Seed corn }			Drying, 5 %;		
					Extraction, 25 %		
Sugar beet	Beet (washed), 6 tons				Sugar not extracted from pulp, 15 %	Sugar, 750 kg.	29 (including tops)

Table 5. Number of adult human subjects who can be supplied with 1 year's requirement of calories and nutrients from the vegetable and animal production of 1 acre of agricultural land

Crop	Yield	Tryptophan			Lysine	Methionine	Calcium	Aneurin	Ascorbic acid	By-products for stock feeding	
		Calories	Calories	Calories						Vitamin A	Available
Vegetable:	18 cwt.	2.4	2.4	2.4	1.8	2.1	0.3	3.8	0.2	0.1	Available
	6 tons	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Available
	5 tons (ware)	3.3	1.7*	1.7*	3.6*	—	0.8	7.7	24.7	0.1	Very little
	20 tons	0.8	12.7	12.7	9.4	3.1	17.1	8.2	182.5	49.3	Very little
Animal:	200 gal.	0.6	2.8	2.8	4.1	1.5	3.6	0.6	0.7-0.0	1.0-2.0	None
	2 bacon pigs†	0.5	1.1	1.1	2.0	0.6	0.5	2.2	0.2	1.0	Available (cereal straw)
	½ fattening animal	0.4	1.5	1.5	3.0	0.9	0.4	0.1	0.2	0.7-1.2	None
	2400 (20 birds)‡	0.2	1.4	1.4	0.8	1.5	0.3	0.5	0.0	0.7-1.2	Available (cereal straw)

* Based on analysis of isolated protein.
† Including purchase of 0.5 cwt. fish meal.
‡ Including purchase of 1 cwt. fish meal.

The following papers were consulted in constructing the table: Andross (1946), Booth, Carter, Jones & Moran (1946), Halnan (1944), Kon (1946a, b), Lampitt & Goldenberg (1946), Leitch (1944), Leitch & Godden (1941), Lockwood (1945), McCance & Widdowson (1946), Orr (1944), Pyke (1946), Watson & More (1942), Wood & Newman (1928).

Some crops leave little waste. Potato haulms are not edible under most conditions, and the only waste products are small unsaleable potatoes and a variable recovery of potato peelings in swill. To utilize these, the pig is normally used, and, as The Nutrition Society has often been informed, the pig is not a very efficient converter of food. Where cellulosic materials are available, the cow is the most efficient animal. Table 6 shows the

Table 6. *Contribution of animal products to the human-food production from 1 acre of land devoted to wheat or sugar beet*

(All results expressed in terms of the number of adults that could be supplied for 1 year from 1 acre)

Nutrient	Wheat		Sugar beet	
	Direct (flour)	Indirect (27 gal. milk)	Direct (sugar)	Indirect (134 gal. milk)
Calories	2·4	0·1	3·2	0·4
Tryptophan	2·4	0·4	0	1·9
Lysine	1·8	0·6	0	2·7
Methionine	2·1	0·2	0	1·0
Calcium	0·3	0·5	0	2·4
Aneurin	3·8	0·1	0	0·4
Ascorbic acid	0·2	0·1	0	0·5
Vitamin A	0·1	0·1-0·2	0	0·7-1·4*

* According to season of year.

Table 7. *Equivalent in terms of milk of the waste products from 1 acre of agricultural land of the sugar-beet industry*

	Starch equivalent (kg.)
7 tons tops, with 25 % wastage, starch equivalent 8 %	420
1·5 cwt. dried beet pulp per ton of washed beets = 9 cwt., starch equivalent 61 %	274
Total	694

A cow requires 9300 kg. starch equivalent during her lifetime to produce 1800 gal. milk (rearing and 3 years' milking life)

So by-products from 1 acre sugar beet are equivalent to

$$\frac{1800 \times 694}{9300} = 134 \text{ gal. milk}$$

conversion of waste products from the wheat industry and the sugar-beet industry into human food. These calculations were based on the starch equivalent supplied by the by-products and the fact that the cost of rearing a cow and her production of 1800 gal. milk involves feeding her with 9300 kg. starch equivalent. The amount of milk produced from by-products is then calculated by proportion as shown in Table 7. The utilization of the waste products of these two crops by animals provides a very large additional supply of nutrients. With wheat, the milk produced from the bran and tail corn supplies almost sufficient lysine to make good the deficiency of lysine of the 75 % extraction flour and more than doubles the calcium supply.

In conclusion, therefore, in dealing with the conversion of plants and animals into human food it is not with their comparative merits that we have to deal but with their complementary merits. An agricultural system devoid of livestock is not an efficient method of using our land nor is a system based in its entirety on livestock production.

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Economic and Statistical Aspects of Vegetable and Animal Foods

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In deciding what balance of vegetable and animal foods shall be available to the population of this country the relative nutritional and gastronomical values of the foods have to be weighed very carefully against what is economically practical. Though we know that it is possible to live and work on a diet containing only a very small contribution of animal protein and fats, most of us would probably work better and live more happily on a more mixed diet containing a larger amount of animal foods. The balance between the two types of food is largely determined by three factors: the acreage of land available for agriculture, the size of the agricultural labour force, and the extent to which we can rely on imported food. In this paper, I shall first indicate the relation of these economic factors to the physiological aspects of vegetable and animal foods, and then give some indication of how far home agriculture can be expected to make a greater contribution to our food requirements.

Physiological efficiency

Since to a considerable extent animals eat foods that could be utilized for human consumption or use land that could be cropped with cereals or potatoes, a measure of the efficiency of conversion of feeding-stuffs to human foods by animals is essential. There has been in the past some disagreement on the most appropriate measure to use; it will be as well therefore to consider first what is the best measure of efficiency and how it should be obtained. The yield of protein expressed as a percentage of the amount of protein fed has been widely used as an index of physiological efficiency; but this would only be a satisfactory measure if protein was the chief factor limiting further production. In fact, though lack of protein may be of major importance on some individual farms, particularly poultry farms, the livestock production in general is restricted not by lack of protein but by the available energy supplies. Sufficient protein for dairy cattle and other stock can be provided from home-grown foods and the ration of concentrates. The principal measures of efficiency that are of interest are therefore the ratio of proteins, fats, vitamins and other nutrients produced per unit of energy supplied in the feed. If the protein supplies of the population are critical, the first measure will be of greatest interest, but if fats are also short the ratio of the total energy produced (available to human beings) to the energy supplied may provide an adequate overall index.

In making assessments of this kind it is not sufficient to take the conventional allowances for maintenance and production for the adult animal. With the dairy cow, for example, allowance must also be made for the food required in the production and rearing of the calf up to the beginning of lactation, for maintenance during the dry period and for the food required to fatten her off when she leaves the dairy herd. On the credit side will be the milk produced in the course of, say, 5 years of productive life and the food value of the carcass. Similar calculations may be made for other types of livestock. These give the efficiencies shown in Table 1.

Table 1. *Efficiency of conversion of food by farm animals*

Kind of stock	Energy produced (as percentage of available energy in food)				Protein produced (as percentage of protein equivalent fed)
	As protein*	As fat*	As carbo-hydrate*	Total	
				Present data* 1917 data†	
Dairy cows	3·7	11·1	4·6	19·4 15·0	23
Beef cattle	1·6	5·2	—	6·8 6·7	10
Sheep	1·3	7·5	—	8·8 10·4	11
Pigs	1·8	16·7	—	18·5 17·9	12
Fowls	3·0	6·7	—	9·7 7·7	32

* Quoted from Yates & Boyd (1949), except that revised values are given for sheep.
† Royal Society (1917).

Similar figures for total energy produced, prepared during the 1914–18 war (Royal Society, 1917), are also shown for comparison.

Though there are considerable differences in efficiency between the different farm animals, Table 1 shows that, regarded merely as converters of energy, all animals are decidedly wasteful. In round figures, it takes ten units of energy (available to the livestock), to produce one unit of energy available to human beings. On the energy : energy basis dairy cattle and pigs are outstandingly more efficient than the other livestock; on the protein : energy basis dairy cows again lead, closely followed by fowls. It will be seen from the last column of the table that the chief effect of the inappropriate protein : protein ratio is to overvalue the contribution of fowls relative to other livestock.

Some economic considerations

There are important qualifications to be noted in using these purely physiological measures of efficiency. Farm animals, particularly herbivores, can utilize foods that are of no value to human beings. Beef cattle and sheep come out very low on each assessment; but to a considerable extent they are the product, as stores, of the hill and marginal land often unsuited to any other branch of farming.

Another qualification arises from the differences in yield/acre of the different crops, resulting in varying land requirements for the different forms of livestock. Estimates of yield/acre are given in Table 2. It is not entirely satisfactory to use

Table 2. Yield in cwt./acre of available energy and protein from different crops

Crop	Starch equivalent	Protein equivalent
Cereals:		
Grain	14	1·7
Straw*	2	0·1
Potatoes	28	1·3
Kale	27	4·2
Grass:		
First-grade ryegrass pasture	20-40	3·0-4·0
Second-grade pasture	13-19	1·8-3·0
Poor agrostis pasture	6-12	0·6-1·8
Rough grazings	0-5	0·0-0·5

* Assuming that three-quarters of the oat straw and one-quarter of the wheat and barley straw are utilized.

average values, since some parts of the country, owing to soil and climatic conditions, are more suited to one type of crop than another. The yield of grass is particularly variable, owing in part to these factors but mainly to variations in management; a series of estimated yields is shown to indicate the range. The relative yields of cereals and grass are of particular interest; they show that good grass in a suitable district can yield fully half as much again as cereals, and at the expense of less labour. Indeed it is possible by heavy nitrogen dressings for grass drying or storage to push up yields of grass very much further. Thus if grass can be made to yield twice as much as cereals the dairy cow will produce $2 \times 19\cdot4\%$, or nearly 40% , of the energy value of a cereal crop.

A third qualification to be noted in the data of Table 1 arises from the very different labour requirements of the different forms of livestock. Estimates of the man-hours/cwt. starch equivalent produced are shown in Table 3.

Table 3. *Labour requirements of farm animals and crops*
(Man-hours/cwt. starch equivalent produced)¹

	Direct requirements	Total labour requirements*
Animal:		
Dairy cows	24	30
Bullocks	12	15
Sheep	10	12
Pigs	5	23
Fowls	24	52
Crop:		
Wheat	3	—
Sugar beet†	8	—
Potatoes	7	—

* Including labour requirement for harvest and storage of feeding-stuffs.

† Assuming 12 % sugar yield.

Dairy cows have twice the labour requirements of bullocks, per unit of energy produced, and rather more than twice the requirements of sheep. Pigs, which Table 1 shows to be relatively efficient on the energy : energy basis, absorb relatively little direct labour, but most of their food has to be harvested and stored; moreover, the foods themselves are to a considerable extent directly consumable by human beings. The hen is expensive in direct labour, and in the main requires stored foods.

The second part of the table shows that for equal amounts of available energy produced the labour requirements for growing and harvesting cereal crops are far below the labour requirements for animals; the farm labour requirements of root crops are about half the total labour requirements for bullocks and sheep.

Sufficient has been said to show the limitations to which estimates of purely physiological efficiency are subject when we are considering the supplies of vegetable and animal foods from the wider economic angle. The construction of a table similar to Table 1 but in terms of economic efficiency would, however, be a formidable task. Thus we would have to balance the relative values of land units given by Table 2 against the labour units of Table 3; to these we would have to add similar complex problems affecting, for example, rent and capital. A simpler and more direct approach is possible by considering the selling price of the different livestock products. Within the country the prices of the different crop and livestock products, although fixed by negotiations between the producers and the ministries, are likely to bear sufficient relation to actual costs of production to form a rough overall integration of the different factors already discussed. The prices paid to the farmer in relation to the energy produced (£/cwt. starch equivalent) are shown in Table 4. On the basis of farmers' prices the cost of energy produced by the dairy cow is well below that from other livestock. At the same time, now that liquid milk supplies are adequate, it would be reasonable to expect any additional supplies to be converted into cheese or butter,

thereby substantially increasing the cost per unit energy. Fowls and beef cattle, though expensive on the energy basis, come very close to milk on cost per unit protein. No allowance has been made for wastage or distribution costs, which will affect milk and eggs more seriously than meat, or for the value of hides and other by-products which will reduce somewhat the costs for cattle, sheep and pigs.

Table 4. *Cost of energy in vegetable and livestock products*
(£/cwt. starch equivalent, 1949-50 prices)

	Farmers' price	Import price
Animal:		
Dairy cows (milk)	8.8	—
Beef cattle	12.2	6.4
Sheep	13.8	6.8
Pigs	10.3	7.2
Fowls (eggs)	15.2	10.5
Crop:		
Cereals	2.0	1.9
Potatoes	2.8	—
Sugar beet*	2.2	—

* Assuming 12 % sugar yield.

Costs of energy from crops for human consumption are also shown in Table 4. No allowances have been made for processing of cereals and sugar beet, or for the high wastage of potatoes. In round figures, it appears that one unit of available energy from livestock is four to six times as expensive as a unit from crops.

As a matter of interest, import prices are also given in Table 4; the prices are comparable subject to the quality considerations in favour of the home product.

Current agricultural production

In the light of the foregoing discussion, it is of interest to consider briefly the amount and nature of the total food supplies of the country. In Table 5 are shown the energy

Table 5. *Energy equivalents of total available food supplies of the United Kingdom, 1945**

	Acreage (acres × 10 ⁶)	Starch equivalent (tons × 10 ⁶)		
		For man	For animals	Total
Home-produced foods:				
Vegetable: Tillage crops	13.8	3.2	5.7	8.9
Temporary and permanent grass	17.2	—	12.7	12.7
Rough grazing	17.3	—	0.9	0.9
Total	48.3	3.2	19.3	22.5
Animal	—	1.9	—	1.9
Manufacturing by-products	—	—	1.4	1.4
Imported foods (net imports):				
Vegetable	—	4.6	1.0	8.6
Animal	—	3.0		
Total	—	12.7	21.7	34.4

* Most of the estimates in the table are those given by the Central Statistical Office (1950), but some details are from unpublished data supplied by the Ministry of Agriculture.

values of home-grown foods going directly to human beings and to livestock; the table shows how the total supplies for man are divided between home production and imports and between vegetable and animal foods.

Home produce in 1945 supplied about 40% of the total energy requirements of the population, 25% coming direct from crops and 15% through the animal. The ratio of home production to imports was much the same for vegetable and animal sources. Although the output of home-produced animal foods was barely 40% of our requirements, the animals consumed 86% of the total energy available.

The quantity of vegetable foods for human consumption in 1945-6 was almost double the prewar figure, and this level of production has been maintained in the post-war period. The output of animal products has recovered from the low figures of the war years and in 1949-50 was a little above that of 1936-8; the reduction in pigs and sheep was more than outweighed by the increase in milk. There was a considerable reduction in the importation of animal feeding-stuffs. If allowance is made for this, the net output shows an increase over prewar values of one-third by 1945-6 and of almost one-half by 1949-50.

The arable side of our farms is in the main now running at a high level of productivity, and no large increases can be expected from this source without cutting into the large acreage at present devoted to livestock. Though greater efficiency appears to have been partly responsible for the increase in dairy production, there is no doubt that further increases in efficiency are possible. Since so large a part of our resources is devoted to livestock production, it is clear that the considerable increases that are possible in the productivity of grassland, together with better utilization of the fodder provided and better stock management, could lead to an appreciable improvement in human nutritional standards.

Although, as we have seen, it is quite common for well-managed grass to give 25 cwt. starch equivalent per acre, the estimated yield in Table 5 is no more than 15 cwt. starch equivalent per acre. The whole process of utilization of home-grown foods could also be made materially more efficient. Thus from Table 5 it may be calculated that only 9% of the energy value of foods supplied to livestock is returned in the form of animal products for human consumption; this indicates that the actual efficiency attained is 20-25% lower than the theoretical values given in Table 1.

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Nutritive Value of Vegetable Proteins and its Enhancement by Admixture

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Introduction

It is only since the beginning of the present century that different proteins have been accredited with different nutritive values. Previously, the proteins in a diet represented purely the necessary nitrogenous fraction, and were 'proteins' and nothing more. The conception of differing 'biological values' possessed by different proteins was founded on the experimental work of Max Rubner and his assistant Karl Thomas (1909). Their investigations and later those of Mitchell (1923-4) showed the differences existing in the amounts of different proteins which were required to maintain an adult human subject or animal in nitrogenous equilibrium or to support the growth of a young animal. The support of growth in the growing organisms is a more searching test for the nutritive value of a protein than the maintenance of an adult, and this criterion was adopted by Osborne and Mendel in their long series of investigations. (See Osborne, Mendel & Ferry, 1919). By their method the relative nutritive values of different proteins were measured by the weight increase of the experimental animal, usually the young rat, corresponding to the ingestion of a given amount of a protein, which provided the sole source of nitrogen in the diet. The different diets compared contained the same proportion of protein, which was less than the optimum amount in an otherwise complete ration and was thus the factor limiting growth. The ratio, $\frac{\text{g. weight increase}}{\text{g. protein ingested}}$, known as the P.E.R. or 'protein-efficiency ratio', has proved to be a useful measure of nutritive value.

A new approach has been developed from the knowledge of the chemical composition of proteins. As Osborne & Mendel (1914) said: 'The question of protein synthesis has now become a problem of the biochemical department of amino-acids.' Differences in nutritive worth have been found to correspond to differences in the amounts and relative proportions of the different amino-acids which they contain (see Mitchell & Hamilton, 1929), and the differences are particularly important as regards those amino-acids that are not synthesized in the animal organism, but must be supplied in the dietary proteins (Rose, 1938).

There are advantages and disadvantages in all the methods mentioned above for studying the nutritive efficiency of proteins (Chick, 1947) but, in general, the results obtained by the different methods have shown a satisfactory degree of concordance (Block & Mitchell, 1946-7).

Proteins of animal origin generally contain a more satisfactory mixture of amino-acids than those contained in vegetables, which are usually relatively poor in tryptophan

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and lysine, amino-acids essential for support of the growing animal. In a mixed diet, however, great advantage can be secured by an appropriate mixture of proteins from different foods, whereby a deficiency of an essential amino-acid in one may be corrected by an excess in another. This complementary action exists between many animal and vegetable proteins, and is well illustrated by the observation that mixtures of animal proteins from meat or milk with those from cereals have shown a growth-supporting ability not inferior to that of animal protein alone (see McCollum, Orent-Keiles & Day, 1939; Hoagland & Snider, 1927; Mitchell & Carman, 1926). The reason is obvious. Cereals are well supplied with most of the essential amino-acids, but are deficient in the essential amino-acid, lysine, which is relatively abundant in the proteins of meat or milk.

*Examples of enhancement by admixture of the nutritive
value of vegetable proteins*

Beef tea and toast. An interesting example of such a complementary effect came under observation in the Division of Nutrition of the Lister Institute during a series of tests on the capacity of the proteins of some common foods to support the growth of weanling rats. The hot aqueous extract known as beef tea, from which the solid matter had been removed, was formerly recommended as a nourishing food for invalids and convalescents. In later years, however, it fell into disrepute, well-informed opinion arguing that all nourishment had been removed with the coagulated material and that the resulting beef tea was no more than a pleasant and stimulating drink. In experiments with weanling rats, fed on a ration in which the nitrogen was provided from beef tea, no weight increase occurred, even when the nitrogen was present to the extent of 3.6% (20 % 'crude protein') in the diet. When, however, in a diet containing only 2.2% nitrogen, one-quarter was supplied by the beef tea and the remainder by white flour, steady growth occurred and at a rate greater than when white flour alone supplied all the nitrogen (Chick & Slack, 1945). The effect was easily explained. About 70% of the nitrogen in the beef tea was in the form of gelatine, an imperfect protein lacking both tryptophan and cystine, both of which are present in satisfactory amount in wheat proteins. Gelatine, on the other hand, is rich in lysine, an amino-acid in which cereal proteins are notably deficient. This sparing action for protein is a well-known attribute of gelatine (see Lusk, 1928). Jellies and beef tea, taken with a little toast or bread and butter, can, therefore, be safely trusted to provide convalescents with a pleasant and nourishing snack.

Wheat fractions. Complementary action can be demonstrated also between the different vegetable proteins contained in different foods and sometimes in the same food. An example of the latter is the relation between the proteins contained in the outer layers (bran) and the inner portions (endosperm) of the wheat grain. A diet in which the protein was derived in equal amounts from the bran and endosperm of the same sample of wheat was found to maintain growth at a rate exceeding that which occurred on diets supplying an equal proportion of protein from either the bran or the white flour alone (Chick, Cutting, Martin & Slack, 1947). The supplementary action in this case is explained partly by the fact that the proteins of bran are richer in

tryptophan and lysine than those of white flour (Jones & Gersdorff, 1923-4, 1925; Barton-Wright & Moran, 1946), but what the special contribution of the endosperm proteins may be is at present unknown.

Potato protein and non-protein nitrogen fraction. Another example of a complementary action between different nitrogenous materials in a single food is found in the potato. In this tuber the non-protein nitrogen, which amounts to about half the total, has a marked supplementary effect for the potato protein (tuberin). With diets of comparable nitrogen content, better growth was obtained when it was supplied by the whole tuber than when derived from the purified potato protein (Chick & Slack, 1949). The non-protein fraction, as contained in potato sap from which the protein had been separated by heat coagulation, was incapable of promoting any weight increase in the experimental animals, but was found to have a markedly stimulating action when combined with wheat gluten, a protein which has the lowest nutritive value of the mixture of proteins present in wheat. With a diet containing 20% gluten (on dry weight) as source of protein, the average weight increase of weanling rats was 6.5 g. weekly over a period of 7 weeks. When one-quarter of the gluten was replaced by an equivalent amount of nitrogen from the non-protein nitrogenous fraction of potato sap, the growth rate was nearly doubled, to an average weight increase of 11.5 g. weekly. In this instance no explanation has been found in terms of amino-acid supplementation. Only about one-fifth of the non-protein nitrogen of the potato consists of amino-acid nitrogen, and analyses of this fraction have not shown an excess of any essential amino-acid that might so greatly improve the nutritive value of wheat gluten (Slack, 1948).

Cereals and soya-bean meal. Perhaps the most impressive instance of supplementation between vegetable proteins is shown by mixtures of cereal proteins with those of the soya bean, where the mutual advantage gained is great enough to produce a nutritive value comparable with that of milk proteins. In experiments with young rats on diets containing about 10% of protein derived from white wheat flour or soya flour or milk, the figures for the protein-efficiency ratio were found to be, respectively, 0.83, 2.14 and 2.84. When, however, the 10% of protein was supplied by a mixture of soya and white flour proteins in equal amount the protein-efficiency ratio was about 2.0 and about equal to that corresponding to a similar mixture of soya and milk proteins (Hove, Carpenter & Harrel, 1945; see also Jones & Divine, 1944).

A preparation of soya flour and malted cereals was produced by Caprino in Rome in 1944 and found to be an acceptable food for young children at a time of great milk scarcity. In the expectation that such foods might find useful application in Central Europe in the period following the recent war, the Division of Nutrition of the Lister Institute acceded to the request of the relief organization of U.N.R.R.A. to make an experimental study of such foods. To our surprise we found that a diet containing a mixture of soya flour, malt extract and white flour, in which these ingredients supplied, respectively, 56, 34 and 10% of the total protein, was about equal in growth-supporting value to a diet in which the same amount of total protein was provided entirely from milk (Chick & Slack, 1946). Following this result, similar combinations of soya and cereals have received extensive trials in Germany since the end of the war, which are

described by Dean (1949, 1951). The particular value of soya in these combinations lies in the unusually high proportion of lysine contained in its proteins. Its presence can make good the deficiency of this important amino-acid in the proteins of the cereals and in most other vegetable proteins that have been investigated. When the results of trustworthy analyses are available of the proteins of other legumes and of root and other vegetables, sources may be found of proteins as rich in lysine as those of soya. At present we seem to be dependent on some admixture of soya in any attempt to produce a food of high nutritive value from vegetable products alone.

The animal protein factor

It would appear, therefore, judged by the practicability of providing a satisfactory supply of essential amino-acids, that it is possible to replace animal proteins in a human diet by an appropriate combination of vegetable proteins. On these grounds alone, there would seem to be no scientific basis for the conviction, widely held, that a certain proportion of animal protein is necessary in a human diet, a conviction which will be strengthened by the evidence Dr Wills has collected in her work among undernourished young children in tropical and sub-tropical countries (Wills, 1951).

The question, however, arises whether the need for animal protein may not, in fact, be the need for some special nutrient, some animal protein factor, not itself of a protein or amino-acid nature, which is usually found to accompany the protein in animal foods.

Cary and co-workers described experiments in which young rats receiving a diet containing 25 % protein (5 % from yeast and 20 % from casein) failed to grow normally if the casein had been purified by successive extractions with hot alcohol (Cary, Hartman, Dryden & Likely, 1946; Hartman, 1946). Normal growth was, however, secured by addition of various animal foods or of a small amount of liver extract, but the necessary factor was not present in yeast, or in the cereals, legumes and root vegetables investigated. The essential nutrient in liver extract was later found to be identical with the anti-pernicious-anaemia principle now known as vitamin B₁₂ (Hartman, Dryden & Cary, 1949).

The presence in the ration of a source of vitamin B₁₂ also has been found essential for satisfactory nutrition of poultry and hatching of their eggs (Ott, Rickes & Wood, 1948; see also Stokstad & Jukes, 1949; Snell & Wright, 1950).

Vitamin B₁₂, now regarded as identical with an essential 'animal protein factor', is known to be capable of storage in the animal organism, and it is probable that mothers that have been fed on a generous mixed diet may transmit substantial reserves to their offspring. It is significant that, in order to demonstrate the need of weanling rats for this nutrient, Cary and his colleagues found it necessary to deprive the mothers during the period of lactation. In the experiments described above, with weanling rats fed on diets containing combinations of vegetable proteins, the mothers had received an excellent mixed diet up to the time of weaning their young, and the fact may explain the lack of evidence in these tests that the young animals had need of any 'animal protein factor' in their ration.

Whether the previous diet of the human mother may influence the capacity of the human baby to subsist on a purely vegetable diet for a given period after weaning is a point of interest worthy of study.

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* Given in original as Gartman, obvious misprint.

The Clinical Picture in Children Fed after Weaning on a Predominantly Vegetable Diet

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Introduction

The clinical findings in infants and children fed after weaning on predominantly vegetable diets is the subject of this paper; my experience has been chiefly with the African, so most of the space will be devoted to a description of the syndrome of malignant malnutrition, kwashiorkor, or fatty liver disease of infants as Waterlow calls it, as seen in the Bantu race. It must be understood that this syndrome, which I take to be the extreme expression of the ill-effects of such diets, is not limited to the African; it is seen in all races, and Czerny's 'Mehlnährschaden' is probably the same condition in European children.

A study of populations living on diets consisting predominantly of carbohydrates suggests that there are three very constant ill-effects on the body. The first is on the haemopoietic organs; children and adults are either anaemic or have a tendency to develop an anaemia, generally macrocytic, when under stress. The second effect is on the liver; the lack of protein in the diet is associated with the development of fatty livers in children and with a high incidence of cirrhosis and malignant disease in the adult. The third effect which apparently results partly from the lack of protein itself and partly from the impaired liver function is a shortage of suitable protein so that the body has to call on its own supplies, as is shown by muscular wasting and by an abnormally low level of serum albumin; with the last is associated a tendency to develop oedema. Further, unless the diet contains a suitable mixture of vegetable products, there is often a high incidence of deficiency states due to inadequate supplies of the vitamins of the B₂ complex, skin lesions being especially frequent.

The clinical material studied

The material studied consisted of thirty-six consecutive cases of kwashiorkor admitted to McCord's Zulu Hospital, Durban, under the care of Dr Frank Walt (Walt, Wills & Nightingale, 1950). The diagnosis was based on what we considered to be the essential clinical signs: anorexia, wasting particularly of the muscles which might be masked by oedema, an enlarged liver, and oedema associated with low values for serum proteins. Skin lesions, though not essential for diagnosis, were present in most of the cases.

The children varied in age from 3 months to 6 years. The histories, always unreliable, were depressingly uniform: the baby had been breast fed and then weaned on to a standard diet of *samp* (Kaffir corn) and mealie-meal porridge; no fresh milk or only minute quantities, no meat, eggs or fish and only the very occasional green vegetable. Generally, the mother said that the child had been well until a short time before when there had been an attack of diarrhoea followed by oedema. Sometimes there was a history of several previous attacks, and one atrophic case gave a history of severe oedema which had cleared up some time before admission.

The cases were of two types: the commoner was the oedematous, many of the children having gross oedema with anasarca; a few, six in all, were of the second type, and were in the last stages of wasting. These atrophic cases were the most severe; they were seen to pass from an oedematous condition to an atrophic one while under observation, and there seems no doubt that the atrophic state is the terminal stage of the disease. Most of the patients had diarrhoea on admission. Examination showed oedema to be present in thirty-two of the thirty-six cases; in thirty it was associated with an enlarged liver, which we assumed to be related to the existence of fatty infiltration (Pls. 1 and 2). Skin changes were very striking in the majority of the patients, though a few had clear skins. The hair was reddish brown, there was hyperpigmentation associated with areas of depigmentation, and often there were terrible sores and angular stomatitis. Except in one extremely anaemic child, the urine contained no albumin. Unlike Trowell's cases (Trowell & Muwazi, 1945), tropical diseases were absent; there was no case of hook-worm infection.

Haematological examination showed that all the children were suffering from a moderate normochromic, normocytic or slightly macrocytic anaemia, except in three cases, two fatal, where the anaemia was very severe. The serum proteins were of particular interest; they were estimated as a routine by the copper-sulphate method with use of Hoch's formula, $P = 364 (S - 1.006)$, where P is the protein concentration in g./100 ml. serum and S the apparent specific gravity of the serum (Hoch & Marrack, 1945), and also by the biuret method. The mean value for total serum proteins on admission was 4.5 g./100 ml. with range from 3.5 to 5.6 g./100 ml.; in seven instances only, including two of the atrophic ones, was it over 5.0 g./100 ml. The low values for total serum proteins were due to decrease in the albumin fraction, the globulin values being normal. On treatment the rise in the protein level was extremely rapid; it often started on the 2nd day, and by the 16th–20th day the mean level was 7.0 g./100 ml., a value agreeing closely with that of 7.1 g./100 ml. found in a control group of healthy Bantu children.

Three patients were clinically jaundiced, and two of them died within 24 hr. of admission with grossly enlarged and fatty livers.

Findings at autopsy

Limited post-mortem examinations were made on the four fatal cases, two of each type. In all of them the liver showed gross fatty changes; in the two oedematous ones, both under 2 years of age, the organ weighed 1 lb. and 1 lb. 2 oz., respectively. One of the atrophic cases had a small liver, suggestive of early cirrhosis. The pancreas showed no pathological changes. Two of the patients had died from terminal bronchopneumonia, the other two apparently from liver failure.

Treatment

The treatment was very simple. We argued that the children were dying of protein starvation and excess carbohydrate, and that with such fatty livers administration of fat was contra-indicated, so we decided to force-feed them with *maas*, which is curds made from skim milk, and with *maas* only, to which, after a week, skim milk powder was added; later a full mixed diet supplemented with extra milk was given. Improvement was rapid in most cases. After a few days a marked diuresis set in resulting in a rapid loss of weight which was followed by a steady gain. The diarrhoea generally ceased in a few days with no other treatment. The liver gradually decreased in size. The serum proteins increased steadily but the blood count fell slightly at first and then rose slowly, the results being similar to those observed by Altmann (1948) and by Walters, Rossiter & Lehmann (1947) in returned Indian prisoners of war. The last workers showed the fall in the red-cell level to be due to an increase in plasma volume. If a similar increase in plasma volume explained the fall in red-cell level in our cases, the rate of protein regeneration must have been even more striking than the figures suggest, as we never observed a fall in serum protein level once treatment had begun.

The crude death rate in our series was 11 %, or 2.8 % if all deaths within 24 hr. of admission are excluded as was done by Altmann (1948) in his calculation of death rate. The latter figure may be compared with a corrected value of 20 % for Altmann's series

and of 6% for the series of cases treated with hog's stomach by Gillman & Gillman (1946). The death rate for oedematous cases was low in Altmann's series and in ours, being 4.5 and 3.3%, respectively. It is the atrophic cases that are so fatal, and the number of such in any series will determine the death rate in that series.

Discussion

In my opinion the essential causative factor in this condition is associated with a deficiency of animal protein. That animal protein rather than vegetable protein is essential is not proven, but the evidence in favour of its being so is suggestive. The syndrome occurs in many lands with varied national diets, the common factor in all being a lack of animal protein and an excess of carbohydrate in the diet, and very frequently, though not invariably, a rather low supply of calories. Whipple and his colleagues in their classical experiments showed that for protein regeneration, animal proteins favour the formation of serum albumin, and vegetable protein, with the exception of soya-bean protein, that of serum globulins (McNaught, Scott, Woods & Whipple, 1936). Milk is the best known curative agent.

If the liver lesion is the primary one and a deficiency of protein the essential causative factor, it is natural that lipotropic factors such as methionine and choline should have been considered as possible curative agents. Gillman & Gillman (1945) and Waterlow (1948) used the substances with no success. Vitamins, too, have been tried and found wanting. Milk is the basis of successful treatment.

Taylor & Chhuttani (1945) studied the incidence of anaemia among Indian troops of whom 17,000 were meat eaters and 1188 were vegetarians, all living under good conditions, with a low sick rate. The men had been on army rations for 2 years. They had received the same basic rations which yielded about 3000 Cal. and 80 g. vegetable protein but the meat eaters received an additional ration of 6 oz. fresh mutton with bone daily, the vegetarians one of 4½ oz. tinned milk 3 times a week, and ¾ oz. ghee and 3 oz. atta or rice twice a week, but the milk was frequently not taken. The incidence of anaemia severe enough for admission to hospital was at the yearly rate of 58.5/1000 for macrocytic anaemia among vegetarians, and of 2.6/1000 for normocytic or microcytic, hypochromic anaemia among meat eaters. The difference was even more striking in the incidence of anaemias with red-cell counts of 2 millions or less or a haemoglobin value below 5.8 g./100 ml.; among vegetarians the rate was 24.7/1000, and among meat eaters 0.16/1000. Anaemia, especially macrocytic anaemia, is one of the most constant findings among peoples who depend for their protein on vegetable sources. Indian troops on active service had a far higher incidence of anaemia than British troops exposed to the same risks of malaria and other tropical diseases: the meat eaters suffered as well as the vegetarians, since their meat ration, which had to be fresh owing to supply difficulties, might reach them only once a fortnight (Marriott, 1945). Indian prisoners of war suffered even more severely from macrocytic anaemia, as their diet was extremely poor, often completely vegetarian and low in calories.

The evidence for the occurrence of protein deficiency in adults on predominantly vegetable diets is more circumstantial. It is idle to look in adults for the same syndrome as in children, since the adults of those populations where the incidence of malignant





malnutrition is high in the children will themselves have suffered the same stresses in their infancy. One could expect, therefore, that the liver lesion would progress in any individuals who had failed to recover completely from malignant malnutrition in infancy. In the children's departments of the hospitals in the West Indies and South Africa, one sees small patients, generally boys, with every grade of liver damage from an enlarged hard liver to the final stages of advanced cirrhosis. In those countries and in India routine post-mortem examinations and hospital records provide the evidence of a very high incidence of cirrhosis and carcinoma of the liver; the incidence is far higher than among Europeans and the lesions occur at a much earlier age. True malignant malnutrition also is found among adults, and Trowell & Muwazi (1945) quote a figure of 10% of all admissions to the Uganda Medical School Hospital at Kampala.

The clinical picture in populations weaned on to and existing on diets predominantly or almost entirely of vegetable origin includes underdevelopment, poor physique, anaemia, liver damage associated with cirrhosis and malignant disease, abnormal values for serum proteins with a tendency to develop oedema, and often all the signs and symptoms of vitamin B₂-complex deficiencies. Given a diet with an inadequate supply of calories derived mainly from one or more staple carbohydrates and with little or no animal protein, and malignant malnutrition will appear with all its sequelae. That adequate calories from mixed vegetable staples can prevent the syndrome and give maximum health has yet to be demonstrated.

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EXPLANATION OF PLATES

Pl. 1. Bantu child, aged about 18 months, with the oedematous form of kwashiorkor, showing severe generalized anasarca and moderate skin lesions.

Pl. 2. Head of same child as in Pl. 1. Note pale, scanty hair and severe oedema of face.

Both plates by the courtesy of the Senior Pathologist, Central Pathological Laboratory, Durban, South Africa.

The Nutritional Adequacy of a Vegetable Substitute for Milk

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The problem of supplying a substitute for milk can be resolved into the separate problems of finding substitutes for the various components: minerals, carbohydrate, fat, protein and vitamins.

The supply of minerals seems to be the easiest to deal with. Sodium must always be added to a vegetable diet, calcium nearly always, and iron usually. Carbohydrate forms the greater part of most plant foods, soya being a notable exception, and here the problem is to alter the carbohydrate into a form that is easily digestible. There are many vegetable sources of easily absorbed and well-tolerated fats. The fat requirements of man are unknown. It may be necessary to supply small quantities of unsaturated fatty acids, but fats can be synthesized in the human body, even by children. The chief importance of dietary fats seems to lie in their high calorific value and, since most vegetable foods are bulky in proportion to the calories they provide, the inclusion of fats is usually an advantage. The vitamins of milk are seldom regarded as among its most important constituents, and I want to be excused from discussing most of them. Dr Chick (1951) and Dr Wills (1951) have emphasized the importance of protein, and I am going to discuss principally the possibilities of replacing the proteins of milk.

Fairly convincing evidence could be brought forward, from what we can deduce of the food habits of our prehistoric ancestors, from the pattern of our dentition, from the comparatively recent establishment of agriculture, to show that through the ages the proportion of animal protein in the diet of man has undergone considerable reduction. Now we have a disease, kwashiorkor, malignant malnutrition, which may be the result of the reduction of the animal protein to below the minimum compatible with life, at least, with life at a time of rapid growth.

There can be few races or even individuals who exist on diets that are exclusively vegetable in origin. There must surely be only a handful of vegetarians who deny themselves or their children milk or cheese or an unfertilized hen's egg. Some races, who are obliged by scarcity to live on a vegetarian diet, eat meat in an orgy on the rare occasions when they can get it. Others add insects, ants, caterpillars and locusts (dried locusts are 50% crude protein) to their diets when they can, especially when there is no meat or fish available. It is probable that if the whole, not merely the largest items, of the diet of any race of fine physique were analysed, it would be found to contain a set of amino-acids resembling more or less closely that in milk protein, or in the protein of meat or fish.

It seems then that we are being ambitious if we attempt to provide a good diet from none but plant sources. In spite of this, I intend to confine myself to the problem of feeding young children on such diets. My own experience has been mostly with children. They probably exaggerate the adult's need for amino-acids (just as the rat, which grows even more rapidly, exaggerates the child's needs), and we know how great is the necessity of providing a cheap and adequate transitional diet, to fill the gap between breast milk and the adult diet. In this country we use for this purpose large amounts of cow's milk and increasing amounts of cereals. Is it possible, in less fortunate countries, to do without the milk? Has anyone succeeded so far? Success must have been achieved, but there are very few reliable scientific records. Most of them, as Dr Chick (1951) has said, related to diets which included soya-bean products. Tso (1928, 1929) reared a few children in the 1st year of life on diets in which soya milk, and a small amount of egg, provided the protein. The children thrived, but had to be given extra calcium if rickets was to be avoided. Lane (1931) weaned a pair of

twins on to a truly vegetarian diet at the age of 5 months, using a milk made chiefly from soya, almonds, peanuts and wheat flour. These children also thrived, and continued to do so for the whole period of observation, which lasted nearly 3 years. The experiments of Rittinger, Dembo & Torrey (1935) are well known. They fed infants on various soya milks with satisfactory results, and could not detect any improvements when they added a little dried skim milk. One of these experiments was an apparently unrecognized trial of protein supplementation; a malt syrup was used to supply carbohydrate, but may have supplied also 20–30% of the total protein. Cereals were added to the diets of most of the children at the age of 12 weeks. Soya milks have been given also to Indian infants, and have in some experiments been thought not far inferior to cow's milk, but the reports of the experiments have always been given in an incomplete form, so that the weight gains cannot be properly assessed. Soya preparations are used extensively in the United States for feeding children who cannot tolerate cow's milk, but when Stoesser (1944) tried one of the most widely advertised of these preparations, his results were very poor. The food caused gastro-intestinal upsets in most of the children, and it was obviously unsuitable for use as a permanent diet. We can only hope it has been improved by now.

Willemin-Clog (1930) investigated the use of sunflower-seed meal in infant feeding, and had a fair measure of success, although she noticed that sunflower as the only source of protein was not, apparently, adequate for more than a few weeks. One of her collaborators (Ribadeau-Dumas, 1946) has more recently expressed the opinion that the vegetable proteins are best considered as 'milk-sparers', although a mixture of rice, barley and sunflower was a valuable food for children.

Dr Chick (1951) referred to mixtures of malted cereals, barley and wheat, with soya which she found could produce good growth in rats. I have already given this Society a survey of the results of our use of similar mixtures in child feeding (Dean, 1949). Our first discovery was that there were unexpected and very important difficulties in the way of manufacture. One whole batch of mixture which we thought had been made in the same way as Dr Chick's most successful mixture had to be abandoned because it caused diarrhoea. To-day, I am going to give some additional details of our use of more successful mixtures. In these, 25% of the protein was derived from barley, 5% from wheat and 70% from soya. In Dr Chick's best mixture of these three ingredients, 34% of the protein was derived from barley, 10% from wheat and 56% from soya.

Our two mixtures were exactly the same except that one of them (mixture B) contained soya that had been steamed only long enough to remove the bitter material, whereas the other (mixture C) contained soya that had been steamed for 100 min. to remove also the trypsin inhibitor.

The children, who were between 1 and 2 years old, and were living in a rather overcrowded orphanage, were divided into two equal groups. One was given a simple diet, semolina, vegetables (mostly potato), butter, and some bread, supplemented by cow's milk; and the other group had the same basic diet supplemented by one of the cereal-soya mixtures. The milk and the mixtures were made into a pudding with the semolina. All the children were liberally provided with vitamins A, D and C, and we made sure there was no shortage of calcium.

In the milk group, about 33% of the calories were obtained from fresh whole milk, but in the other group about 50% of the calories came from the cereal-soya mixtures. Of the total calories in the milk diet 11% were protein calories, and of these 60% were derived from milk; in the cereal-soya diets, 13% of the total calories were protein calories, and 70% of these came from the mixtures. The results of the trials are given in Fig. 1. Trial 1 lasted 16 weeks, and trial 2, 8 weeks. The periods are short, but we

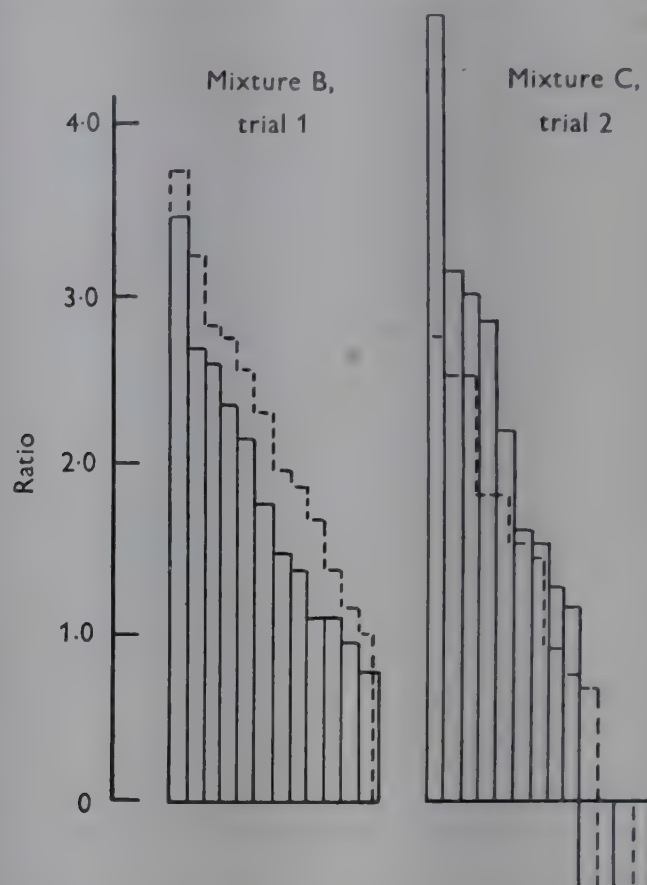


Fig. 1. Ratios of actual to standard weight gains of individual children given cereal-soya mixtures B (in trial 1) and C (in trial 2). The dotted lines indicate the ratios of the children in the contemporary control groups, who were given a diet containing fresh whole milk.

were quite sure that during these periods the children on the cereal-soya diets had no animal protein of any kind. Nearly all the children exceeded the standard gain in weight. There was an epidemic of infective diarrhoea in trial 2 which affected children in both groups. Some of the trouble, however, may have been due to mixture C. We were unable to find the exact cause, but it was probably over-heating during the spray-drying process used for large-scale manufacture. Trial 2 and some later trials made us decide that the removal of the trypsin inhibitor increased the nutritive value of the soya. The approximate amounts of the essential amino-acids in the mixtures, and in the complete diets were calculated from the data of Block & Mitchell (1946-7). When the amounts were compared with the amounts of the same amino-acids in human milk, on the basis of equal amounts of total nitrogen, the differences were small, but the essential amino-acids represented about 63% of the total nitrogen in the human milk, and only 47% of that in the cereal-soya mixtures. This was one reason why we found it necessary to give a higher proportion of protein in the cereal-soya diets than in the milk diets.

It seems justifiable to conclude that if the mixture of amino-acids is suitably adjusted, a diet entirely of plant origin can give excellent results, at least for short periods, in the feeding of children. Dr Chick's (1951) work on young rats is, in fact, applicable to young human beings.

We have sought to extend the scope of our work to other plant materials, and, following Willemin-Clog's example, we have studied sunflower-seed meal, which is a rich source of protein and seems a useful alternative to soya. The soya bean is not universally available, and it cannot, for instance, be grown well in this country, whereas sunflowers can. We have also, not entirely by chance, taken an interest in maize. Sunflower protein contains twice as much of both lysine and tryptophan as maize protein, and we have found that rats grow excellently on various combinations of sunflower and maize, especially if a little yeast is added to the diets.

The success of these experiments has made us speculate on the possible cause of kwashiorkor. We know the disease can be cured by milk. It appears also to have been cured by various other substances which contain animal protein, serum given intravenously, liver and hog's stomach. Does the disease occur because of amino-acid deficiencies in the diet, a single or multiple deficiency of one or more of the amino-acids I have mentioned or of some other, and is it cured by proteins which contain large amounts of those amino-acids? There is another, and quite different, possibility which also has occurred to us as a result of our experiments. We were anxious to find out if our cereal-soya mixtures were improved by the addition of vitamin B₁₂. It is not present in soya or in any of the cereals we have used in our child-feeding experiments. When we added it to the cereal-soya mixture B, we found that it had an effect on the growth of rats as beneficial as the addition of quite considerable amounts of milk protein, and we hope to have an opportunity in the near future of demonstrating a similar effect in children. There is a further point worth consideration. Vitamin B₁₂ has not been demonstrated in maize, but it is, I believe, to be found in all those substances which can cure kwashiorkor. This may be merely a coincidence, but a trial of vitamin B₁₂ seems to be indicated, not only on these empirical grounds, but because the vitamin is believed to be of importance in transmethylation and therefore in the utilization of lipotropic substances such as choline and methionine.

We think we have evidence enough to show that it is possible to evolve diets containing protein exclusively from plant sources, which will successfully rival diets containing fair amounts of animal protein. This circumlocution is preferable to the use of the term 'milk substitutes', because so many diets do not contain milk. We are really only at the beginning, however, of knowing how best to apply our experience. There are many diverse factors that have to be taken into account. They include variations in the amounts of amino-acids in our raw materials, depending on the strain of wheat or yeast, for instance, or the method of cultivation of rice; alterations in biological value caused by heating and by the reactions between amino-acids and sugars, reactions which may escape detection by the ordinary methods of analysis; variations in the methods of preparation of foods which may impair digestibility and cause loose stools, as our mixture C did; differences introduced by the necessary jump from the laboratory or 'pilot' scale of production to the full factory process, and all

the time we must remember that economic necessity forces us to prepare our new foods in the simplest and cheapest way, and as far as possible from materials locally available.

We cannot be experts in all the sciences simultaneously involved. We rely on the co-operation of the paediatricians, the biochemists, the agriculturalists, the cereal chemists, the educators, the administrators, even the politicians.

We know there are millions of undernourished children. We believe they could be better nourished if we used our plant resources more perfectly. We must find means of translating our belief into fact.

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Vitamin C Reserves of British Troops in England and Scotland during the Winter and Spring, 1941-2*

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The object of this work was to study the vitamin C status ('reserve') of the Army in relation to its bearing upon the prevalence of (a) gingivitis, and (b) scurvy or antecedent conditions and symptoms.

EXPERIMENTAL

Plan of investigation

The general plan of the investigation was drawn up by Major-General D. T. Richardson, C.B., M.C., K.H.S., Director of Hygiene, as follows:

Comparisons were instituted between recruits with less than 1 month's service, as indicating the condition of the civilian working-class population, and soldiers of over 1 year's service. Should a considerable difference be shown to exist, it might be argued that it would be unwise to accept a standard of nutrition in the Army which was demonstrably below that of civilians, especially as the troops were liable to face hardships and severe exercise, which, combined perhaps with a further lowering of nutritional standard, might be expected to lead to the risk of outbreaks of scurvy among those with low reserves.

It was decided that a hundred men should be examined in each Command, in winter and late spring, namely:

Western and Scottish Commands: soldiers who had been stationed in an industrial area for 6 months or over.

Northern and Southern Commands: soldiers who had been stationed in an agricultural area for 6 months or over.

Eastern Command: recruits who had come from civil employment in an industrial area.

South-eastern Command: recruits who had come from civil employment in an agricultural area.

Where possible, fifty soldiers were taken from a field ambulance which had a reception station, where the standard of messing was presumably good. The remaining fifty were to come from an infantry unit.

In the final arrangements the men who volunteered for the tests were as follows:

Northern Command: infantry and field ambulance men stationed in a country district in Norfolk.

* The investigation was undertaken upon instructions from the Director-General, Army Medical Services, Lieutenant-General Sir Alexander Hood, K.C.B., C.B.E., M.D., K.H.P. Permission to publish has been granted.

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Southern Command: infantry and field ambulance men in Hampshire.

Western Command: pioneers in a town, Crewe.

Scottish Command: infantry and R.A.M.C. personnel of a military hospital in Glasgow.

Eastern and South-eastern Commands: recruits, as arranged, in Essex and Aldershot districts, respectively; the latter had been in the Army less than 2 weeks.

Operational procedure for the test

General. The test selected was that described by Harris & Abbasy (1937) in which the vitamin C status is ascertained by giving large daily doses and analysing a sample of urine collected 3–5 or 6 hr. after administration, and noting the number of days necessary for saturation. When the vitamin is excreted in quantity, saturation is said to be reached or approximated to. The method has been used extensively, standardized and critically examined (see, for example, Harris, 1942, 1943; Nutrition Society, 1945).

The men received 0.75 g. synthetic vitamin C, L-ascorbic acid, daily till saturated. The urine was collected and analysed; the volume was measured and the total amount excreted in the period calculated, since it is upon this—and not the concentration in the sample—that the test depends.

The work in each Command occupied a week, and the Commands were visited in the same order—South-eastern, Eastern, Southern, Western, Northern, Scottish. Starting on 2 December 1941, the winter tour ended on 17 January 1942.

To see how the troops had fared during late winter and spring, when green foods were scarce and potatoes had lost much of their vitamin C, a second tour was begun 4 months later on 13 April 1942 and ended on 24 May.

The tests were carried out by the author, who did all the chemical analyses. The preliminary work was done in the Royal Army Medical College, Hygiene Laboratory, then at Mytchett, under the late Colonel C. S. Ryles, O.B.E., and Major Stanley Elliott, O.B.E., T.D., F.R.I.C., to whom the author is much indebted for help and advice.

Period of retention. The period of retention, 3 hr., was sometimes found inconveniently long, and when shorter periods had to be taken, a rough correction was introduced on the assumption that the volume was a rectilinear function of the time, which is probably approximately true if the time is not too long.

In the spring series, a period of 2 hr. was rigorously adhered to. Though giving strictly comparable results as between the different Commands, this may have led to a systematic error on the high side, since the peak of excretion is about 4 hr. after dosing. It was considered that this was unlikely to be serious. In the winter, saturation was taken to be approached with an excretion of 50 mg. of the vitamin, but in the spring with 35 mg. for the shorter period.

Measurement of vitamin C. Pure L-ascorbic acid was used to adjust and check the concentration of the 2:6-dichlorophenolindophenol reagent at each station.

In the winter series the volume of each sample of urine was measured and 10.0 ml. were pipetted into a bottle containing 1.0 ml. glacial acetic acid. Thus for each sample

a calculation had to be made to convert the concentration into the actual output of vitamin.

Furthermore, concentrated and turbid urines were troublesome to titrate. In the spring series, samples were made up to 0.5 or 1 l., using tap water, and this did not introduce any error. Thus each burette reading corresponded to a definite quantity of vitamin, and much time was saved. Since in all about 5000 samples were titrated, the reduction in labour was considerable, as compared with the standard technique. The end-point of the titrations was also better.

RESULTS AND DISCUSSION

Volume of urine passed

The volume of urine passed after 3 hr. retention varied in the winter series from 0 to 1090 ml., being for the South-eastern, Eastern, Southern, Western, Northern and Scottish Commands, respectively, 0-1060, 6-460, 80-1090, 50-750, 15-660 and 65-1025 ml. The average volumes for the first ten samples of each Command were 320, 90, 290, 360, 220 and 410 ml. The urines of low volume in the Eastern Command were mostly turbid and difficult to titrate. This was occasioned by the issue of an order, only discovered by the investigator after 2 days, that the men should not drink or have their dinner during the 6 hr. period. The men of the South-eastern and Eastern Commands were recruits and gave quite similar results, so it did not appear that the much smaller volume of urine had introduced any serious difference in the quantity of vitamin excreted. It was not possible to ascertain the range of urine volumes over which the time of retention is related to the quantity of vitamin excreted irrespective of the volume passed. It may have been wide, for on the 1st day soldiers W 59 and W 60 passed, respectively, 520 and 50 ml., yet the ascorbic acid excreted was 6.7 and 7.5 mg. Some men consistently passed little or no urine and had accordingly to be classed in the lowest group. They were advised to drink more.

Normal effect of dosing with vitamin C

Table 1 shows the excretion of vitamin C after each dose, either measured or calculated for a period of retention of 3 hr. These results are typical.

In some instances there was an immediate excretion of over 50 mg. in 3 hr., commonly accepted as denoting saturation. More usually for 2 or 3 days little or no change in output occurred. Thereafter, more or less suddenly, there was a rise. Unfortunately, the four doses allowed for in the winter series were too few in number to determine when saturation occurred in those men whose vitamin level was low, and these 112 men (19 % of the total, omitting casualties) were divided up so that those showing an approach to saturation were classed as becoming saturated after a fifth dose, though they never received it. Those who, though still low, had almost doubled their initial excretion were classed as 5A. Several of the graphs suggest that these, taken as saturated after six doses, would in fact have been saturated after five. Those classed as 5B showed little or no response after five doses. For graphic representation, they were taken as saturated after seven doses, though some might have been saturated after six, possibly even after five, and some might have required

over seven. It is important to give doses adequate to classify the most resistant, as these are the ones likely to give trouble. The Scottish tests on personnel of the Royal Navy showed that at least five doses were necessary (McNee & Reid, 1942).

Table 1. *Daily excretion of vitamin C by seventeen subjects receiving a daily dose of 0.75 g. of the vitamin*

No. of subject	Excretion (mg.) on day				Day of saturation
	1	2	3	4	
4	61	100	—	—	1
18	81	149	—	—	1
7	11	72	—	—	2
29	1	113	—	—	2
8	3	13	88	—	3
98	3	2	96	—	3
7 ^x	4	21	230	—	3
10	2	4	34	127	4
97	2	8	22	71	4
94	2	3	9	176	4
79	6	3	4	16	5
61	4	5	7	18	5
21	3	7	14	12	5?
39	0	0	2	4	?
11	0	0	5	0	?
4 ^x	8	6	7	4	?
32	2	2	5	5	?

In the spring series, up to five doses were given to recruits and to the Southern Command men. Up to six doses were given in the Western, Northern and Scottish Commands; and the hard residue in the last should have had seven or more doses but, owing to vomiting, the doses were discontinued after six. The classifications 6, 6*A* and 6*B* also 7, 7*A* and 7*B* were similarly used for indicating residues after five and six doses respectively, in the same way as already explained when four doses were given.

Effect of dosing beyond the accepted value for saturation, and some abnormal cases

Table 2 shows that, when a subject has come to the condition of heavy excretion, further dosing might result in no further increase, or even an appreciable decrease from the maximum, as if a state of tolerance were reached. The maximum excretion was 220–230 mg. in 3 hr.

There were occasional instances of a fall where a rise might have been expected—nos. 45, 16 and 65—and of an almost steady pre-saturation level for a couple of days—nos. 52, 5 and 79. Harris (1940, fig. 1) records, too, several cases of the rise to saturation followed by a diminishing output for 1 or 2 days before this again reached a maximum, but as his outputs are plotted on a logarithmic scale the phenomenon is less noticeable. Dr Harris has since informed me that this occurrence has been frequently noted (e.g. Harris, 1942, 1943).

Saturation tests in the six Commands

The tables giving the analytical results for each of the 1200 men for each day have been omitted. Their use lay in the comparisons with the conditions of the men's

Table 2. *Daily excretion of vitamin C by seventeen subjects receiving a daily dose of 0.75 g. of the vitamin when dosing was continued beyond saturation; also some unusual occurrences*

No. of subject	Excretion (mg.) on day after saturation				Day of saturation
	1	2	3	4	
39x	135	75	—	—	1
94x	80	62	—	—	1
98x	184	92	—	—	1
15	82	83	—	—	1
53	163	117	91	—	1
41	2	62	224	—	2
83	6	60	218	—	2
60	5	111	78	53*	2
45	23	74	54	177	2
95	35	26	194	—	3
16	31	4	72	—	3
89	5	13	149	136	3
52	4	39	36	129	4
92	32	37	48	—	4
5	5	6	43	31	5
79	4	6	30	36	5
65	2	7	25	2	5?

* Small volume (20 ml.) of urine excreted.

mouths as regards gingivitis. The results of the 5000 tests have been summarized in Table 3. The men whose vitamin C reserve was measured were also examined for gingivitis at the same time by the late Brigadier H. Stobie, Consulting Dental Surgeon to the Army, whose report showed that there was no connexion between incidence of gingivitis and vitamin C reserve. This is in agreement with the earlier results of Surgeon Rear-Admiral McNee and his co-workers, obtained on naval personnel (McNee & Reid, 1942).

In using the term 'reserves of vitamin C' no suggestion is implied that the amount present may be without function save as a reserve. Again, some prefer to write of status or level, since vitamin C is not stored in special parts of the body to any considerable degree and it is thought that the test indicates rather the 'degree of saturation' of the tissues.

The data of Table 3 may be grasped more readily by inspection of Figs. 1-3, which show, respectively, the results for men from country and industrial districts, and the total for recruits as against total for soldiers. In these figures a clearer picture is given by plotting not, as heretofore customary, the number saturated after each dose, but the total number that had reached saturation at that period (Atkins, 1943). It may be seen that in each of Figs. 1-3 the full lines, denoting recruits, lie above the broken lines, denoting soldiers, for the same season.

For industrial districts, Fig. 2, and the totals, Fig. 3, the recruits, even in spring, are superior to the soldiers both during winter and spring. Such differences amount to one or two daily doses and even to three for those with least reserve. Furthermore, though almost all the recruits became saturated, a percentage, increasing during winter, of the soldiers failed to saturate. This is shown in Fig. 4. The importance of this

Table 3. *Number of men in South-eastern (SE), Eastern (E), Southern (S), Western (W), Northern (N) and Scottish (SC) Commands becoming saturated after doses as tabulated, also percentage of total for recruits (civilians, SE and E) (C), soldiers (M) and total (T) in winter series (WR) and spring series (SP)*

Cate- gory*	SE		E		S		W		N		SC		C		M		T	
	WR	SP	WR	SP	WR	SP	WR	SP	WR	SP	WR	SP	WR	SP	WR	SP	WR	SP
1	9	0	0	0	2	0	0	0	0	0	0	0	4.5	0	0.5	0	1.9	0
2	31	10	27	10	20	4	7	2	14	5	2	0	29.0	10.4	11.3	2.9	17.4	5.4
3	32	52	47	39	34	14	36	14	43	9	28	4	40	47.1	36.3	10.8	37.4	23.0
4	24	30	15	29	28	38	25	19	24	35	27	30	19	30.6	26.8	32.0	24.3	31.5
5	4	3	6	15	9	17	12	31	9	30	20	28	5	9.3	12.9	27.8	10.2	21.6
5A	0	0	3	0	5	0	10	0	6	0	4	0	1.5	0	6.5	0	4.8	0
5B	1	1	1	0	3	0	8	0	1	0	10	0	1	0	5.7	0	4.0	0
6	0	0	0	3	0	11	0	11	0	9	0	12	0	1.5	0	11.3	0	8
6A	0	0	0	0	0	4	0	0	0	1	0	0	0	0	0	1.3	0	0.9
6B	0	0	0	1	0	7	0	0	0	1	0	1	0	1	0	2.3	0	1.9
7	0	0	0	0	0	0	0	4	0	4	0	9	0	0	0	4.4	0	2.9
7A	0	0	0	0	0	0	0	5	0	2	0	3	0	0	0	2.6	0	1.8
7B	0	0	0	0	0	0	0	6	0	4	0	7	0	0	0	4.4	0	2.9
Casualties	0	5	1	3	1	4	5	4	4	5	9†	1	—	—	—	—	—	—
Total	101	101	100	100	102	99	103	96	101	105	100	95	100	99.9	100	99.8	100	99.9

* See text, p. 277.
† One man, saturated on 2nd day, had been in the country up to the previous week; he has now been included in the casualties.

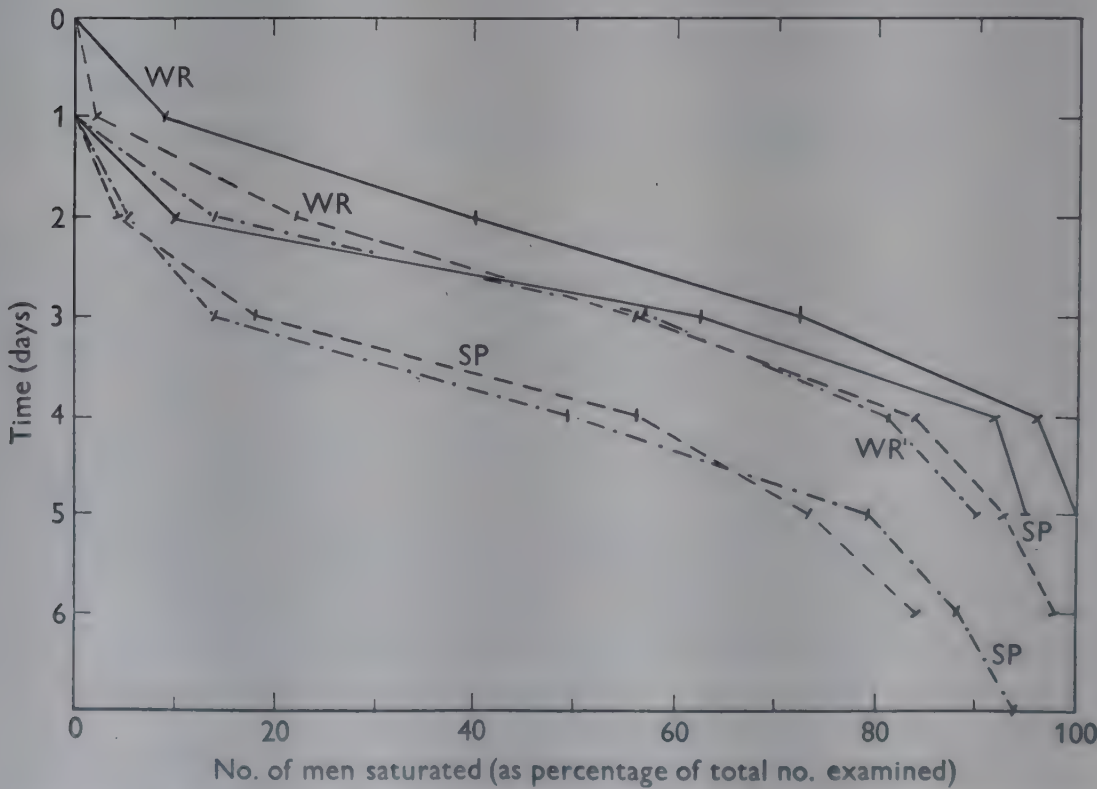


Fig. 1. Time taken to reach saturation by recruits and soldiers from and in the country, in different Commands, receiving a daily dose of 0.75 g. synthetic vitamin C. The graphs show the total number of men who had reached saturation up to the given day inclusive. WR, winter series; SP, spring series; ———, South-eastern Command, recruits; - - - - , Southern Command, soldiers; - , Northern Command, soldiers.

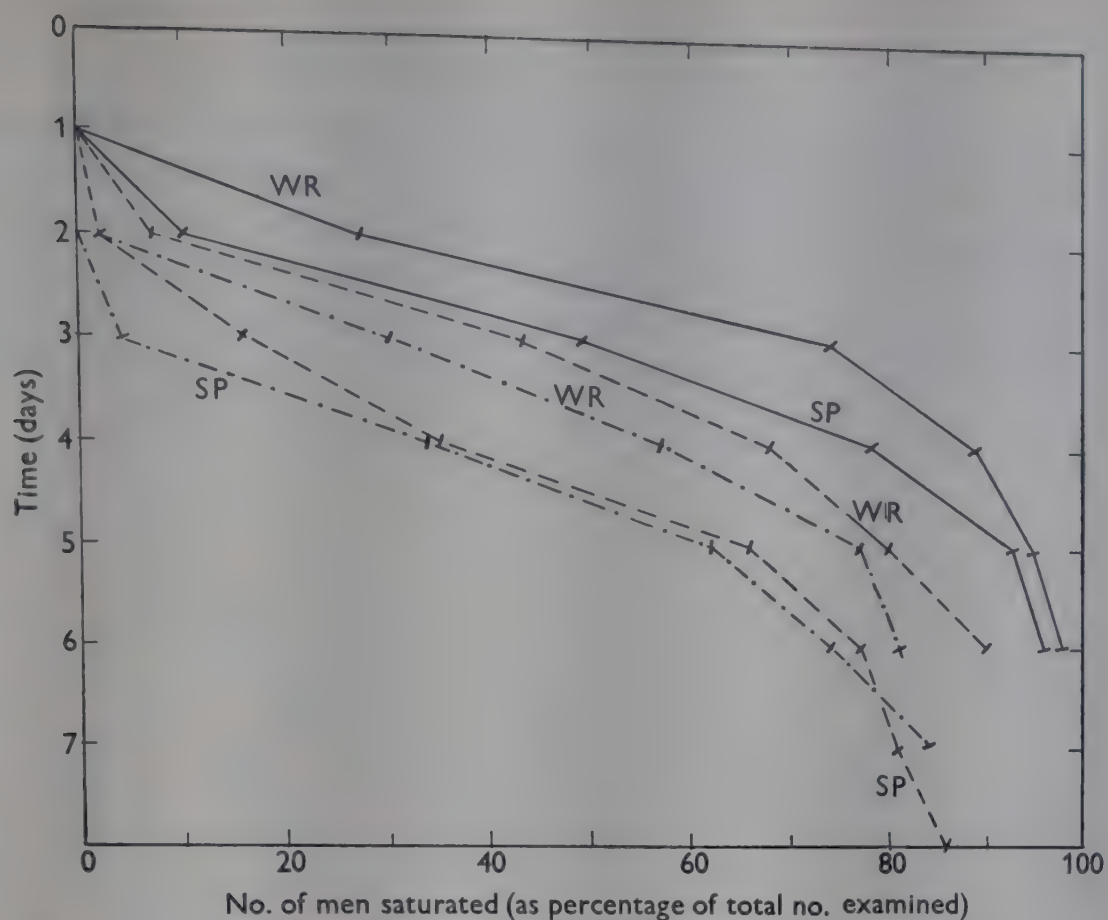


Fig. 2. Time taken to reach saturation by recruits and soldiers from industrial areas, in different Commands, receiving a daily dose of 0.75 g. synthetic vitamin C. The graphs show the total number of men who had reached saturation up to the given day inclusive. WR, winter series; SP, spring series; —, Eastern Command, recruits; ----, Western Command, soldiers; - · - · -, Scottish Command, soldiers.

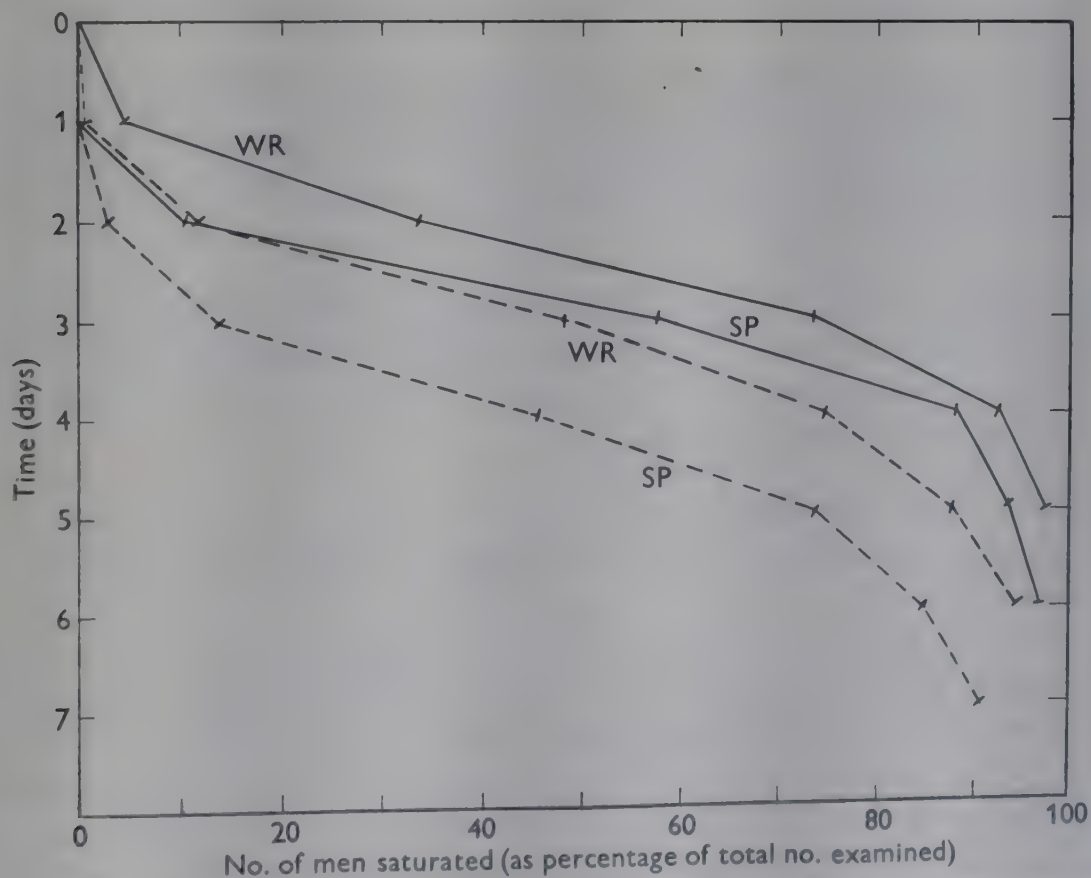


Fig. 3. Time taken to reach saturation by recruits (total examined 200) and by soldiers (total examined 400) from all Commands, receiving a daily dose of 0.75 g. synthetic vitamin C. The graphs show the total number of men who had reached saturation up to the given day inclusive. WR, winter series; SP, spring series; —, recruits; ----, soldiers.

finding lies in the fact that it is this percentage that indicates the potential victims of scurvy and its premonitory symptoms when more severe conditions are encountered; such a percentage, negligible in civilians, is seen to be large in the Army in spring.

In the Southern and Northern Commands, R.A.M.C. and Infantry were used in the trials, all being in the country. In the Southern Command, the Infantry were rather better than the R.A.M.C., but the Northern R.A.M.C. were close to the Southern

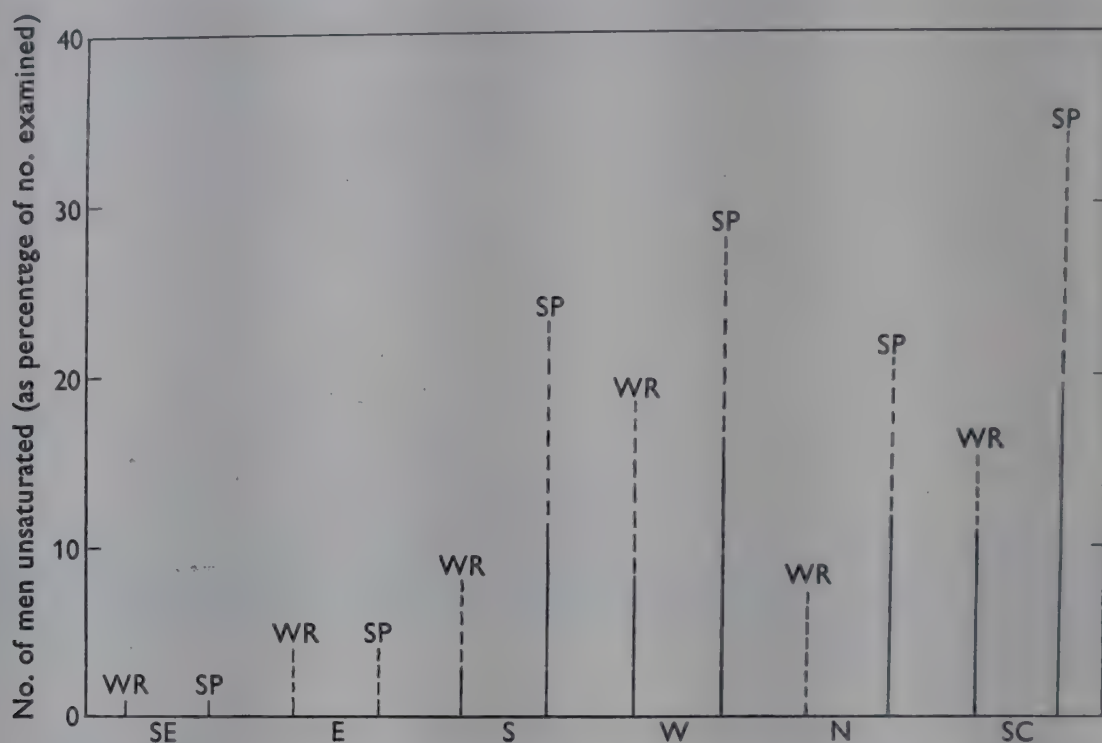


Fig. 4. Number of recruits and soldiers in town and country areas in different Commands remaining unsaturated after five or six doses of 0.75 g. synthetic vitamin C. WR, winter series; SP, spring series; SE, South-eastern Command (recruits, country); E, Eastern Command (recruits, town); S, Southern Command (soldiers, country); W, Western Command (soldiers, town); N, Northern Command (soldiers, country); SC, Scottish Command (soldiers, town); ———, six doses; ----, five doses.

Infantry and tailed off less. The Northern Infantry were decidedly the lowest. They were a normal infantry unit, and probably had more severe training than the Southern, which was a Defence Company. Both R.A.M.C. units were Field Ambulances, and did a considerable amount of marching.

Storage of vitamin C in the body

An attempt was made to get some information on storage within the limits of the saturation-test routine.

In the spring series three men were dosed to saturation and dosed again after omitting the dose for 1 or 2 days. A had no dose on the 3rd and 4th days; one on the 5th saturated him fully. B, with similar omissions, became nearly saturated on the 5th day, and on the 6th day excretion fell to 10 mg. with no dose. D had no dose on the 4th day, but became saturated again when dosed on the 5th. Thus, the excess of vitamin appeared to be quickly eliminated and destroyed, but a saturated man not dosed for 2 days could be restored to saturation by one dose.

It was possible, however, to compare the results for thirty-seven men of an industrial

area, Western Command, who happened to be dosed in both the winter and spring series, respectively from 29 December 1941 to 1 January 1942 and 5 May to 9 May 1942, and also for two men in Southern Command (country) and one man in Scottish Command (city). No change was shown by 30 % in the day upon which they became saturated; 35 % were 1 day later in spring; the remainder were earlier by 1 day (10 %)

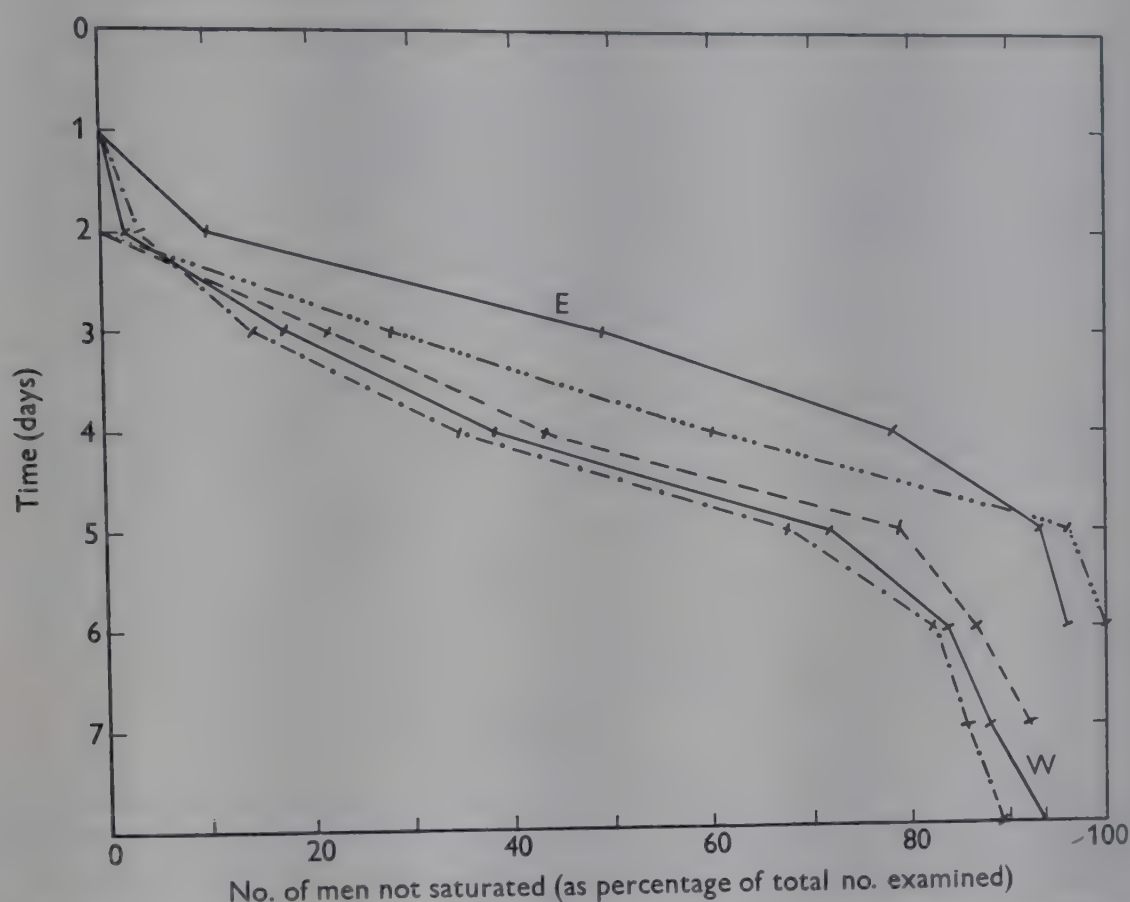


Fig. 5. Time taken to reach saturation in the spring series by recruits and soldiers of Eastern and Western Commands, receiving daily doses of 0.75 g. synthetic vitamin C. The graphs show the total number of men who had reached saturation up to the given day inclusive. — (above), Eastern Command (recruits); — (below), Western Command (soldiers); ·····, Western Command, soldiers saturated 4 months previously; ----, Western Command, soldiers who had received four doses 4 months previously; - · - · - , Western Command, soldiers not dosed before.

or 2 days (5 %) or later by 2 days (12.5 %) or 3 days (7.5 %). There was thus no great difference between these and men not previously saturated, but Fig. 5 shows that the recruits from industrial districts were somewhat superior even to those soldiers who had been dosed to saturation in the winter series 4 months before.

The recruits were markedly superior to the Western Command sample as a whole, and a small but definite difference could be seen to exist between the men previously dosed to saturation and those who had received four doses 4 months previously, though not all of them had become saturated. The lowest in reserves were the Western Command men who had not been dosed in the winter series. Of these, 33 % required over five doses to saturate, as against 28 % for the Western as a whole; 22 % of those dosed before required five doses; but only 4 % of those dosed and saturated 4 months previously required five doses, and only 4 % of the Eastern Command recruits.

Thus, some effect of saturation 4 months previously still remained detectable; alternatively, one might conclude that the metabolism of the men who became saturated easily was in each series detectable by the test, and that they stood out in each series because they used the vitamin more efficiently, or destroyed less of it in the body.

Effect of dosing before and after a meal

In the winter series in Glasgow, fifty men were hospital orderlies, fifty from an infantry regiment. The R.A.M.C. were in sections, A and B, merely for A.R.P. duties, they messed and worked together. The results, however, were very different as between the sections, B reaching saturation sooner than A. This at first appeared to be a severe blow to one's confidence in the method. Inquiries failed to reveal any difference between the sections, such as weight or occupation. The results were reported to the Director of Hygiene at the time without any explanation being possible.

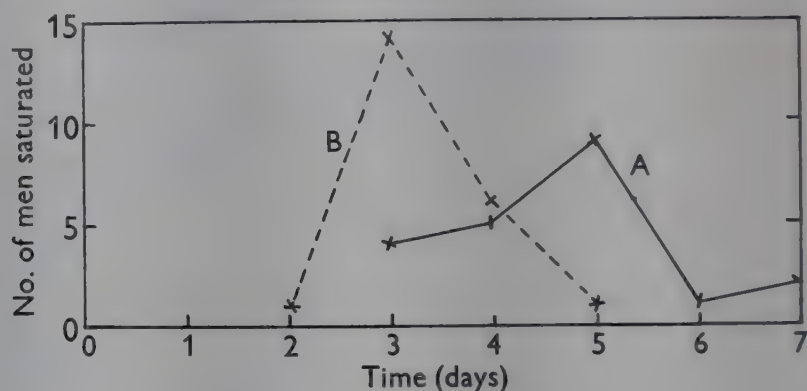


Fig. 6. Time taken to reach saturation by soldiers receiving daily doses of 0.75 g. synthetic vitamin C before (twenty-two men) or after (twenty-one men) breakfast. The graphs show the number of men who had reached saturation up to the given day inclusive. —, section A men, dosed before breakfast; - - -, section B men, dosed after breakfast.

The same hospital was visited 4 months later, and further inquiries elicited the fact that the section A men had paraded at 7.30 a.m. before breakfast, as they had been busy attending to patients. They were dosed at once. The Infantry then arrived, and section B men had breakfast and were dosed afterwards. This routine was followed on subsequent days in order to have an exact repetition from day to day. Thus the vitamin seemed to suffer less destruction and to be better utilized when taken after food. As, however, it appeared unwise to draw a conclusion from a single experiment although fifty men were used, the tabulated results were submitted to statistical analysis and the results were published (Atkins & Fisher, 1944), since the question was not included in the original object of the work.

Four doses were given. As before, those saturated on the 5th day were shown as such, with 5A denoting those likely to be saturated after six doses, and 5B those so low after four doses that no prediction could be made; the latter are plotted as for seven doses. The results are shown in Fig. 6.

To ascertain the probability of the distribution found being due to chance, of the forty-three cases (there were seven casualties out of the fifty) in the two groups to be compared, a line may be drawn between the nineteen saturated in the first 3 days and

the twenty-four others taking longer. One finds in the first group four *A* and fifteen *B*, in the second group eighteen *A* and six *B*. The chance of getting so large a discrepancy, if the numbers in *A* and *B* were really proportional, is about 1:1867. Thus there is no doubt of the statistical significance of the difference observed. The only ascertainable difference between the two groups was that men of section A, who took longer to saturate, had their vitamin C on an empty stomach.

Pathological effects of the doses

Under the term pathological effect one may consider those cases in which the dosing caused, or was associated with, stomach troubles.

(a) *Winter series.* S21 said he always vomited after taking tablets, and he did so on this occasion. No more were given. N101 was much surprised at the effect, almost immediate vomiting. The thirty tablets in a cup of tap water at Mytchett gave a slightly acid taste, the reaction being about pH 3.5.

(b) *Spring series.* E38 refused to take the tablets on the 2nd day, saying that the taste still persisted since he had chewed them on the 1st day. N56 reported that he often suffered from indigestion and used to take kaolin before meals. After four doses he excreted 25 mg., and no further vitamin was given as he complained of a renewal of his indigestion. He was rated as saturated on the 5th day. N86 complained of heartburn and indigestion, which he did not remember ever having had before. The dosing was stopped. He excreted 12 mg. after the fourth dose, indicating probable saturation on the 6th day, possibly on the 5th. SC12 vomited after the tablets on the 5th day, when he excreted 25 mg. He made no complaint, and vomited again on the 6th day, but excreted 42 mg. so must have absorbed a considerable amount of each dose, as he was finally saturated, 35 mg. for a 2 hr. period being taken as the criterion. SC34 vomited on the 6th day and excreted 7 mg., so was classed 7*B*. SC59 also vomited on the 6th day when nearly saturated (excretion 30 mg.), and was grouped as saturated on the 7th day.

On account of the vomiting no further doses beyond six were given, though patients with scurvy may receive up to ten or twelve. Thus McNee & Reid (1942) found that a case of scurvy took eleven 0.7 g. doses, 7.7 g. in all, for saturation and the condition of the gums remained unchanged after 14 days, though the circumfollicular lesions were fading between the fourth and fifth dose.

The thirty-two who had six doses seemed much puzzled as to why they should be so much behind and said they ate potatoes and other vegetables like the rest.

SUMMARY

1. The saturation test of Harris & Abbasy (1937) was applied to recruits, as indicating the condition of civilians, and to soldiers of 1 year's service and upwards, during the winter and late spring 1941-2.

2. The recruits showed a superior vitamin C status compared with the soldiers, so that even in spring their 'reserves' were greater than those of the soldiers as a whole had been 4 months previously in winter.

3. The recruits from the country were slightly superior to those from industrial

areas, and soldiers from country districts had definitely greater reserves than those from industrial areas.

4. These differences become striking when one considers those with the lowest reserves, not saturated even after receiving five or six doses of 0.75 g. L-ascorbic acid. Whereas this was 1 % for the recruits, the soldiers of Scottish Command (Glasgow) showed in spring 34 % not saturated after five doses and 21 % not saturated after six doses. Though no symptoms of approaching scurvy were reported and the men all seemed well, yet exposure to hardships, severe exercise and restricted food might have been expected to evoke scurvy sooner in these men than in the recruits.

5. It would appear unwise to accept as satisfactory a reserve of vitamin C considerably lower than that of the young civilian working-class population. Harris (1943) has shown that the large majority of men receiving the 30 mg. of vitamin C, recommended as the 'standard requirement' of the League of Nations, became saturated in 2 or 3 days; so the soldiers serving in Great Britain had received less during the winter, since large numbers of them were unsaturated after even five or six doses.

6. The evidence available pointed to the desirability of increasing the vitamin C supply of the troops in winter, especially as it was found that forty men saturated in January were only slightly above those not previously dosed, when compared 4 months later in May. Those men previously saturated left a very small unsaturated residue after five doses, namely 4 % as against 33 % for those not dosed before.

7. One test with forty-three men showed that saturation was reached more rapidly in those who had the vitamin dose after breakfast rather than before it.

8. Administration of the vitamin tablets in water resulted in vomiting in two soldiers in winter and two in spring, when also there were two cases of stomach trouble attributed to the tablets.

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Rickets in Sheep

1. The Experimental Production of Rickets in Young Sheep

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Rickets as a widespread disease of children has been recognized since the seventeenth century, and although the therapeutic efficacy of cod-liver oil was recognized even in those days, the precise aetiology of this disorder was not fully determined until about 20 years ago, since it awaited the discovery of the decisive role played by vitamin D in osteogenesis and in phosphorus and calcium metabolism.

In these circumstances it is not surprising that though the existence of widespread osteodystrophic disease in ruminants had been described in parts of Europe (Marek & Wellmann, 1931) and particularly South Africa (Hutcheson, 1895), it was not until Theiler's studies (Theiler, Green & Du Toit, 1924; Theiler, 1934) demonstrated the primary part played by phosphorus that progress was made. Not long afterwards, Du Toit, Malan & Rossouw (1930) and Du Toit, Malan & Groenewald (1931, 1932) were able to show that rickets in lambs and osteoporosis of older sheep ('Lamsiekte') in South Africa were similarly due to phosphorus deficiency.

In Australia, Henry (1912) had demonstrated the value of phosphorus supplements for cattle in New South Wales, and Richardson, Trumble & Shapter (1931) showed the relationship between the low phosphorus content of pastures and occurrence of much ill health in ruminants. It was found, however, that the response to phosphate supplements was often disappointing. The explanation was advanced by Marston (1934) that there was an associated lack of protein in pasture from the phosphorus-deficient areas, sometimes also accompanied by a trace-element deficiency.

In New Zealand the same earlier confusion occurred, as is shown by the accounts of Reakes (1910), Aston (1930) and Wright & Taylor (1931). Though some osteodystrophic lesions encountered were almost certainly due to a suboptimal intake of phosphorus and calcium, much of the unthriftiness of certain sheep-farming areas has since been shown to be due primarily to cobalt deficiency.

The lack of association in South Africa and Australia of rickets or osteomalacia in the ruminant with insufficient vitamin D is hardly surprising in view of the almost universal practice of keeping animals out of doors all their lives, combined with the fact that the latitude of most of the sheep-carrying areas in both countries is such that the incidence of the sun's rays would, even in winter, not be less than 35°. This was claimed by Tisdall & Brown (1929) to be the critical angle below which the anti-

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rachitic effect of solar radiation rapidly diminishes. The South Island of New Zealand and much of Great Britain, however, do not receive sufficient ultraviolet radiation during the mid-winter months for young children, and the importance of vitamin D in the aetiology of rickets in hoggets in New Zealand has come to be recognized. At the same time, rainfall in both countries is more evenly spread, climate more temperate and pasture growth much less subject to drought with its attendant drop in mineral and protein content. The analyses of pasture made by Woodman & Evans (1930) in England and by Elliot, Orr, Wood & Cruickshank (1926) in Scotland show this last point clearly enough.

Under these conditions, various osteodystrophic diseases of sheep have been rather loosely described as 'rickets'. Elliot, Orr, Wood & Crichton (1926), in reporting experiments carried out in Scotland between 1921 and 1924 in which they observed the effect of adding calcium and cod-liver oil to the diet of young sheep, claimed that a rachitic disease, 'bent-leg', occurs spontaneously amongst sheep and is of widespread distribution. Auchinachie & Fraser (1932) were able to produce 'bent-leg' experimentally on a diet deficient in calcium and relatively high in phosphorus (Ca:P ratio = 1:13). Vitamin D alone, given as 10 ml. cod-liver oil daily, was as fully protective as extra calcium. W. L. Stewart (1933) described a disease of sheep prevalent at that time in lambs in Northumberland and Yorkshire and locally called 'cripples', although other names, including rickets, have been given to it. The disorder occurs in lambs from 6 days to 2 months old, with the greatest incidence in animals 1-2 weeks old. The pathological picture appears to be one of deficient osteogenesis, since the bone trabeculas are very slender, the corticalis narrow and osteoblasts few. The evidence from chemical studies of the blood (Shearer & Stewart, 1931) and field experimental work (Piercy, 1934*a, b*) all indicated that this was not true rickets. Similarly, a bone disease of older sheep in Yorkshire, described by Bowes (1932) and known locally as 'cappie' or 'double scaup' because of the thinning of the frontal bones, does not appear to have shown the typical manifestation of rickets. Investigations by Bosworth & Stewart (1932-3) led to the conclusion that the bone lesions were due to arrested osteogenesis associated with porosis.

It had been suggested by Leslie (1935) that the lameness often seen in a proportion of lambs being overwintered in the South Island of New Zealand might be caused by rickets, but it was Fitch (1943) who confirmed the existence of true rickets from a pathological examination of material from a typical case of winter lameness in a hogget in Canterbury, New Zealand. Field trials followed on areas where the disease had been reported to occur to a varying extent each winter, and this led to the discovery that different winter fodder-crops varied in their rachitogenicity, but that young green oats, widely grown for grazing purposes in both New Zealand and Australia, were most actively rickets-producing (Fitch & Ewer, 1944). More rigidly controlled experiments were conducted over the next few years (Ewer & Bartrum, 1948) which clearly established the efficacy of single massive vitamin D dosage both prophylactically and therapeutically and also that the occurrence of rickets in untreated hoggets was always associated with low blood values for inorganic phosphorus but normal values for serum calcium.

Before studying the relationship between phosphorus and vitamin D in the growing sheep, it was necessary to produce rickets experimentally. This communication describes the production of rickets in lambs and the effect of supplementing a rachitogenic diet with vitamin D₂ and of altering the Ca:P ratio.

EXPERIMENTAL

Sheep

In an effort to reduce the variability to a minimum, twenty Welsh lambs of similar age and breeding were selected from a mob of ninety and brought to the Department's farm at Cambridge. They had been born in the latter part of April 1947 and arrived at the beginning of December of the same year and appeared to be in thriving condition. Samples of faeces were taken three times during the following 3 weeks and examined for fluke eggs or parasitic strongylid infestation. No fluke eggs were found and there was only a light nematode infestation (average egg count of 200/g.). However, in view of the interference with mineral metabolism that may occur through chronic worm infestation (Stewart & Shearer, 1932-3; Franklin, Gordon & Macgregor, 1946), anthelmintic treatment was given in the form of a dose of 15 g. phenothiazine on 27 December. On that day the lambs were brought inside and henceforth kept penned indoors with the windows distempered, to be certain that the lambs were not exposed to ultraviolet radiation.

For various reasons, amongst which was the difficulty of getting the sheep to take the experimental diet and of procuring some of its ingredients, the main experimental period did not start until 27 February 1948. Over the pre-experimental period weights were recorded at 3-weekly intervals and blood was analysed for haemoglobin, calcium and phosphorus. The sheep were weighed on 28 January. They were brought fully on to the experimental diet on 27 February and, following weighing on that day, were randomized into five groups of four sheep. The treatment of each group is shown in Table 1.

All sheep were weighed and radiographed, and blood samples were taken at 3-weekly or monthly intervals throughout the experiment. Each group was given its daily ration at 9 a.m. after any residues had been collected and weighed.

Diet

The main requirements appeared to be that the basal diet should be low in phosphorus, relatively high in calcium, contain sufficient protein of high biological value to meet the hoggets' potential growth needs, have enough vitamin A and a minimum quantity of vitamin D. The mixture that was designed to fulfil these needs and formed the daily basal diet given to all the sheep was: dried sugar-beet pulp 600 g., oat-straw chaff 80 g., blood meal 80 g., urea 5 g., molasses 150 g., calcium carbonate 14 g., salt 6 g.

Sufficient sugar-beet pulp and oat-straw chaff were bought to last the entire experiment. The quantities of both these ingredients were twice adjusted during the 1st month to bring the residues to a minimum before they were established at the above

Table 1. *Treatment of experimental sheep*

Group no.	Sheep no.	Treatment
1	4	Low-P, basal diet only
	13	
	16	
	20	
2	7	High-P, basal diet with 15 g. $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ daily
	9	
	11	
	14	
3	1	Low-P* (as group 1) with vitamin D_2 25 mg. calciferol on 27 Feb. 1948; 23 mg. calciferol on 28 May 1948
	6	
	15	
	19	
4	2	Low-P (as group 1) with vitamin D_2 later (sheep nos. 17 and 18 given 25 mg. calciferol on 6 August 1948)
	3	
	17	
	18	
5	5	High-P (as group 2) with vitamin D_2 later (sheep nos. 8 and 10 given 25 mg. calciferol on 6 August 1948)
	8	
	10	
	12	

* The sheep in group 3 were given their first dose of calciferol (equivalent to 1,000,000 i.u. vitamin D_2) dissolved in 10 ml. arachis oil by mouth.

figure. The molasses was mixed with sufficient water (1.5 l.) to moisten the beet pulp and to enable the blood meal to be thoroughly mixed in.

Protein. The complete diet contained 1.21 % nitrogen and, since most of this was from the blood meal, of which the protein may be assumed to contain 16 % nitrogen, the crude protein content of the diet would be 7.6 %.

Phosphorus. The diet was found to contain 0.08 % P, and subsequent measurement of the actual daily intake of P of the low-P sheep expressed as an average figure over the 229 days of the main experimental feeding period was 0.3 g. daily. This is a lower intake than that maintained by the sheep on the low-P diets of Du Toit *et al.* (1932) or Martin & Pierce (1934). It may in fact be considered to represent about one-seventh of the optimum P intake of sheep of this age if the daily requirement of 2.2 g. P suggested by Beeson, Johnson, Bolin & Hickman (1944) be accepted.

Each sheep on the high-P diet was given 15 g. sodium monohydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) daily, equivalent to 1.20 % P in the diet, as a solution in 50 ml. warm water, usually administered on alternate days, except when any of these sheep were being used in a balance trial, when each was dosed daily. From 8 October 1948, the daily supplement was reduced to 7 g./sheep which was equivalent to 0.55 % P in the diet.

Calcium. The basal diet was designed to supply a high intake of Ca. This was done in order to have a wide Ca:P ratio, which has been recognized as being necessary for the establishment of rickets in rats, although Theiler (1934) stated that the production of rickets in sheep is dependent mainly on the amount of P in the diet rather than upon the relative amounts of P, Ca or magnesium. In addition, it was desired to avoid

complication with any of the osteodystrophies of sheep that have been shown to be associated with low serum-Ca values. The condition known as 'bent-leg' (Elliot, Orr, Wood & Crichton, 1926) has already been mentioned. Franklin (1934-5) has shown that a diet low in Ca could cause pathological changes in the bones of sheep which Innes (1934-5) suggested might be regarded as 'rickets'. Moreover, Duckworth, Godden & Thomson (1943), in their study of the development of rickets in sheep where a diet containing 1.6 g. Ca and 1.9 g. P was given, maintained that the most constant diagnostic sign of the onset of the disease was a fall in the serum Ca below 6 mg./100 ml.

The basal diet in the present work was found to contain 1.24 % Ca but, for the above reasons, 14 g. commercial calcium carbonate were added to the daily ration of all the sheep. This brought the Ca content up to 2.37 % (by analysis). In October 1948, when the P supplement was halved, the calcium carbonate was similarly reduced.

Vitamins. It has been shown by Miller, Hart & Cole (1942) and by McElroy & Goss (1940*a, b*, 1941*a, b*) that sheep are able to synthesize in their rumen most, if not all, of the vitamin B complex they require. Similarly, it was made clear by the work of Thomas, La Grange & Culbertson (1942) that the requirements of vitamin E in sheep are met by the common feeds. Guilbert, Miller & Hughes (1937) indicated that the minimum carotene requirement was about 3 mg./100 kg. body-weight, with a recommended allowance of four times this amount.

It is in relation to vitamin A that our diet was probably low, since experimental work concerned with vitamin A deficiency in calves, undertaken at this period by other workers in the Department, in which the same oat-straw chaff was used, indicated that its carotene content was very low. It is, however, well known that sheep normally have a high liver-storage capacity for vitamin A, and Hart (1940-1) showed that efficiency of utilization increases with low intake. It can therefore be reasonably assumed that these experimental sheep had sufficient vitamin A to satisfy their needs for the first 6-8 months. In October the vitamin A content of the blood plasma of the remaining sheep was estimated at weekly intervals, and it appeared that although there was a very wide variation between individual sheep, the trend was fairly sharply downward. For example, between 22 November and 11 January the mean value fell from 86 to 42 i.u./100 ml. A carotene-concentrate solution was then given at the daily rate of 130 i.u. carotene/kg. live weight, and from early March until the end of the experiment this was replaced by vitamin A acetate given in arachis oil at a rate of 20 i.u./kg./day.

The vitamin D content of the diet was of particular interest, and an assay made by Dr W. F. J. Cuthbertson upon an ether extract of 8 kg. of the dried, ground ration as fed gave a value of 0.025 i.u./g. Andrews & Cunningham (1945) found that the vitamin D requirement of young sheep was 160 i.u./100 lb. live weight. From the average intake figures of the sheep receiving different treatments it was found that the diet was theoretically unable to supply the vitamin D requirement of even the high-P animals, and the low food intakes of the low-P sheep would have made this deficiency more serious.

Analytical methods

Blood. Blood samples were obtained from the jugular vein. P estimations were made on duplicate samples of whole blood within 2 hr., using Gomori's (1942) modification of the method of Fiske & Subbarow (1925). Alkaline phosphatase was estimated on the plasma by the method of King & Armstrong (1934) and Ca by the method of Kramer & Tisdall (1921).

Haemoglobin. During the early months of the experiment and until some time after rickets had developed, haemoglobin estimations were made by the alkaline-haematin method (King, 1947), using a photoelectric colorimeter.

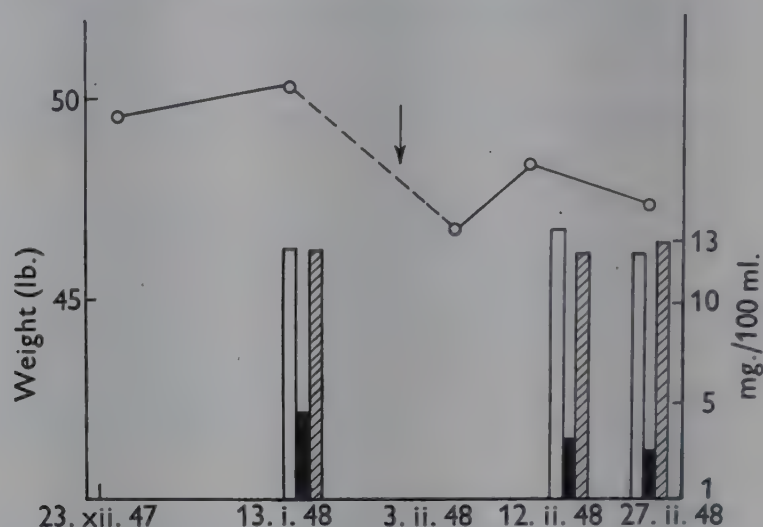


Fig. 1. Mean weights, haemoglobin, blood inorganic-P and serum-Ca values of sheep during the pre-experimental period. ○—○, weight; □, haemoglobin; ■, blood inorganic P; ▨, serum Ca; ↓, shorn.

Pathological examinations. The course of the bone changes was followed by making regular X-ray examinations of the distal epiphysis of the right radius of each sheep and by periodic biopsies of costochondral junctions performed upon representative sheep from the main treatment groups. This latter method was adopted because of the belief expressed by some workers (Follis, Jackson, Eliot & Park, 1943) that the only satisfactory way of diagnosing subclinical rickets is by microscopic examinations of rib junctions.

Tissue phosphatase. Samples of the main organs of rachitic and normal sheep were taken at slaughter, and use was made of Gomori's (1939, 1941) technique to study the distribution of alkaline phosphatase. These results and that of the microscopic examination of the rib junctions will be reported elsewhere.

RESULTS

Pre-experimental period. The effect of the pre-experimental period of feeding upon the weight, haemoglobin values, total serum Ca and blood inorganic P of all the sheep is indicated in Fig. 1, which shows the mean values.

Food consumption. The well-known effect of a low-P diet upon appetite, first noted by Theiler *et al.* (1924) in cattle and subsequently by Du Toit *et al.* (1930) in sheep, soon became apparent. The South African workers, both in the preliminary reports

cited and in their further experiments (Du Toit *et al.* 1932), found that this effect did not become obvious for several months. Martin & Pierce (1934) found that the chaff consumption of their low-P sheep rose almost as much as in their high-P sheep in the early period and began to fall steadily from the 4th month. Stewart (1934-5), although he did not measure the food consumption directly for all sheep, showed that the low-P and control sheep made similar gains over the first 3 months.

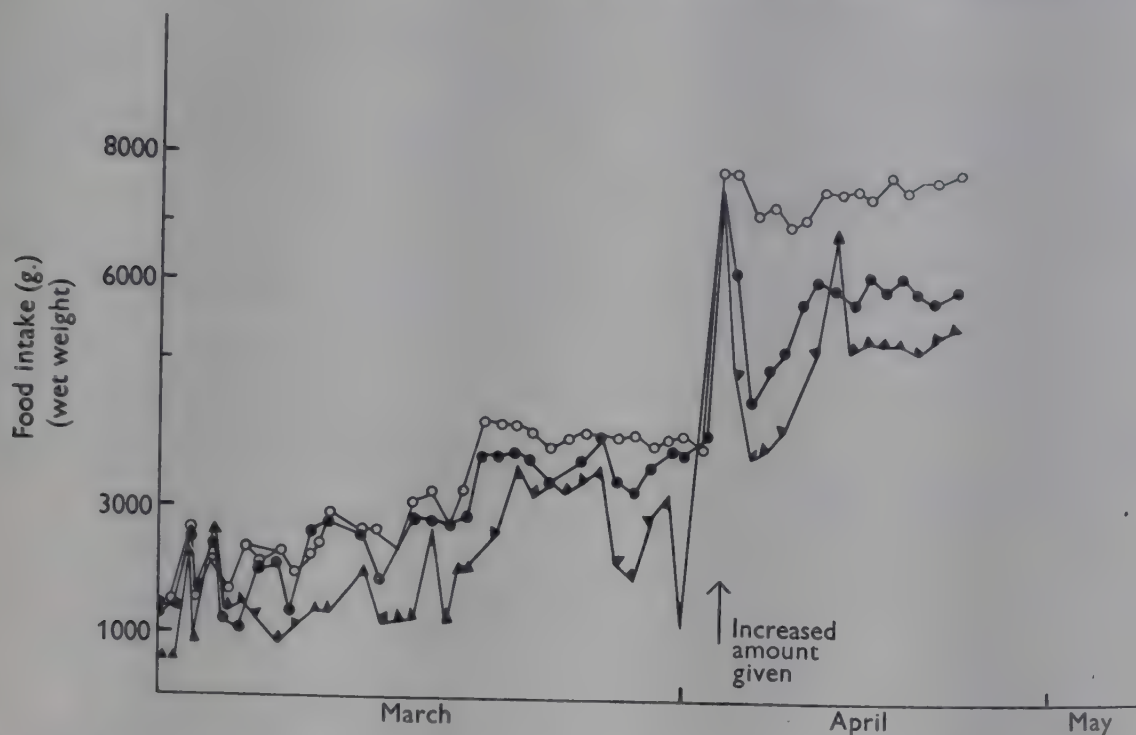


Fig. 2. Net food consumption of the sheep. ●—●, groups 1 and 4 (low-P, high-Ca); ○—○, groups 2 and 5 (high-P, high-Ca); ▲—▲, group 3 (low-P, high-Ca with vitamin D₂).

Within a week of the start of the present experiment the low-P lambs in groups 1, 3 and 4 were consuming less food than the high-P lambs in groups 2 and 5, and this difference became more marked from April onwards (5 weeks after the start of the experiment), when the water added to the beet was increased by 1 lb. This is seen in Fig. 2. The P supplement, in fact, enabled the mean daily food intake to be maintained at approximately 50 % above that of the control sheep in group 3.

The addition of vitamin D in the form of two massive doses of calciferol (group 3) had no beneficial effect on food consumption. Actually the intake of this group was the lowest of all, but it is felt that this can hardly be ascribed to a specific effect of vitamin D, since the numbers in each group were small and the variation in the food consumption of individual sheep in this group, as revealed by balance trials, showed as wide a variation within each of the low-P groups as between the mean values of the groups.

Body-weight. The weight gains naturally reflected the variations in food intake. At the start of the experiment the mean weight of each group was within 1.4 lb. of the total mean weight and the maximum variation was ± 4.2 lb. Fig. 3 shows the weight gains for each sheep over the main experimental period.

Blood composition. The mean values for blood inorganic P soon reflected the

different levels of P intake, and changes also occurred in the serum-Ca levels. The group averages for both elements are shown in Fig. 4.

The tendency, already mentioned, of the blood inorganic P to fall during the pre-experimental period was reversed during the first 10 days of the trial. But after this

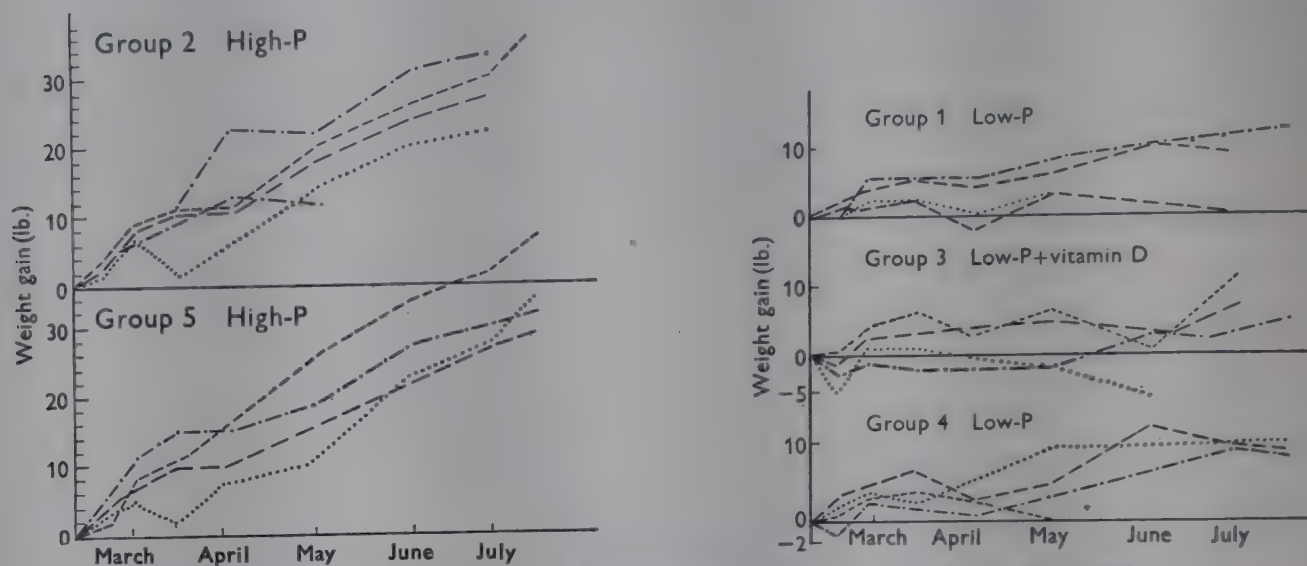


Fig. 3. Weight gains (lb.) of each sheep during the main experimental period.

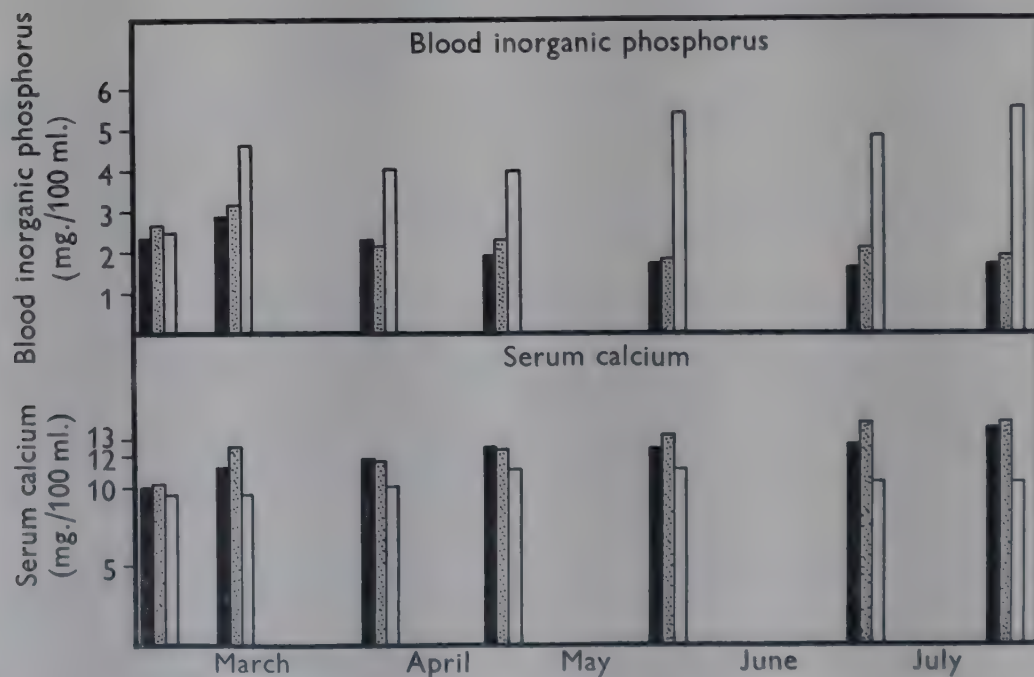


Fig. 4. Mean blood inorganic-P and serum-Ca values of sheep in main treatment groups. ■, groups 1 and 4 (low-P); ▨, group 3 (low-P with vitamin D); □, groups 2 and 5 (high-P).

a severe hypophosphataemia steadily developed in the low-P groups, until the mean group levels in July (6 months from the start) were 1.6 mg./100 ml. in group 1, and 1.7 mg./100 ml. in groups 3 and 4. In the two groups getting the high-P diet, normal levels were reached in the first 10 days and these were maintained throughout.

The effect of the low P intake associated with the high Ca content of the ration was to produce some degree of hypercalcaemia in most sheep in these groups. This

reciprocal relationship between Ca and P in low-P rickets has been noted before (Fraser, 1932; Martin & Pierce, 1934; Stewart, 1934-5).

It soon became clear that estimation of the plasma phosphatase, at least by means of measurement of the reduction of diphenyl phosphate, would not prove a reliable indication of the development of rickets. It was observed that certain sheep in every group consistently gave phosphatase values considerably above or below the mean for the group, so that differences between individuals within some groups were larger than the mean group differences. This confirms the observations made by Duckworth *et al.* (1943) that plasma-phosphatase values in sheep are not of much diagnostic value.

Pathological observations. The rachitogenic capacity of the basal diet combined with the 2 months of confinement indoors was demonstrated by the finding that calcification at the distal radial epiphysis of almost all the sheep had been at least partly arrested by the time the experiment started. An arbitrary system of assessment of the degree of interference with ossification was adopted when examining the radiographs obtained each month. This is shown at the foot of Table 2, which contains the full results of the X-ray examinations until the first experiment with ³²P (see Ewer, 1951).

Table 2. Results of X-ray examination of the right radius of the lambs

Group no.	Sheep no.	Degree of rickets on							
		27.ii.48	22.iii.48	26.iv.48	25.v.48	29.vi.48	27.vii.48	16.viii.48	9.ix.48
1	4	±	±	+	++	++±	++±	++±	+++
	13	+	±	+	±	+±	+	+	±
	16	±	+	+	++	Dead	.	.	.
	20	±	±	+±	++	++±	+++	+++*	.
2	7	±	±	±	-	-	N	N	N
	9	+	±	±	±	-	N	N	.
	11	±	-	+	-	-	-	-	-
	14	±	±	+	-	N	N	N	N
3	1	N	N	-	N	N	+	+ -	++
	6	+	+	-	N	+	++	++	++
	15	±	-	±	+	N	+±	+±*	.
	19	N	-	±	±	N	+	+	.
4	2	-	+±	+±	++±	+±	++	++	+±
	3	-	+	Dead	.	.	.	.	.
	17	N	±	+	++	++±	++	++±	±
	18	+	+	+ -	++	+++	+++	+++*	.
5	5	±	±	N	N	N	N	N	N
	8	-	±	-	N	N	N	N*	.
	10	+	-	-	N	N	N	N	N
	12	±	-	-	N	N	N	N	N

* Killed on 16 August 1948.

N: normal;

+ : mild rickets;

++ : moderate rickets;

+++ : advanced rickets;

- : slight interference with calcification;

± : more marked interference with calcification.

By the end of May all the sheep in the low-P groups 1 and 4 were rachitic, and the extent of the interference with calcification became worse up to July. In group 3, where the sheep had received two large doses of vitamin D₂—at the beginning of the experiment and on 23 May—the increased degree of mineralization was striking. By the end of July, a moderate degree of rickets was discernible in one animal in this

group, and the others were also beginning to show lesions. The antirachitic effect of the vitamin, however, can be clearly seen if the radiograph of florid rickets in sheep no. 4 in group 1 is contrasted with that of a representative (sheep no. 1) of the animals in group 3 (Pl. 1). Weight gains and blood-phosphate values were equally low in both animals. The mild rickets to be seen in most sheep in groups 2 and 5 when the experiment started disappeared as a result of the high P supplement. Normal bone growth was attained by the four sheep of group 5 within 3 months, but for some reason the sheep of group 2 were a little slower in giving a normal picture, although their growth rate was similar to that of group 5. One sheep (no. 11) never gave a completely normal radiograph. Its weight gains were satisfactory but its blood inorganic-P values were below those of the others in the group. In October it exhibited transitory haemoglobinuria associated with inappetence, and a few weeks after its apparent recovery it died suddenly from enterotoxaemia.

A further demonstration of the power of a large dose of vitamin D₂ to influence bone calcification was afforded by studying its effect in sheep already rachitic. Two sheep in group 4 (low-P) and two in group 5 (high-P) were given 25 mg. calciferol in 20 ml. arachis oil by mouth on 6 August. It later became necessary to use one sheep from each group in an experiment with isotopic ³²P but the other two, no. 10 (group 5) and no. 17 (group 4) were kept for further observation. No. 10, a normal sheep, made no obvious response to vitamin D unless its weight gain of 13 lb. during the following 132 days, contrasted with 10 lb. in sheep no. 5 in the same group, is to be regarded as significant. No. 17, a rachitic sheep, made a dramatic response (Pl. 1, 2). On 6 August, the day the vitamin was given, its radiograph was classified as showing moderately severe rickets. By 9 September increased calcification had already occurred to the extent of markedly reducing the intensity of the bone-lesion grading, and by 20 October the sheep was almost normal. It remained radiographically normal until the following February although its blood inorganic-P level was as low as 1.8 mg./100 ml. in December. The sheep also maintained its weight. From February until the last radiograph was taken on 14 July 1949, there was a progressive increase in the width of the uncalcified epiphyseal cartilage until the rachitic lesion was again of moderately severe intensity. Pl. 1, 2 illustrates some of these changes. During the period in which it was free from rickets (6 months or so), this sheep lost all sign of lameness and enlarged knee joints, and even on 1 June it was striking to compare the stiff, restricted gait of no. 4, which had received no vitamin D, with the free, fast walk of no. 17.

Clinical observations. The precise date at which lameness developed in any particular animal on the unsupplemented basal diet was difficult to ascertain with the sheep kept in small pens. A note was made, however, of an alteration in walk, the so-called 'proppy gait', occurring in several animals in groups 1 and 4 by mid-May, about 2½ months from the start of the experiment. Certainly, by 14 August 1948, when all groups were turned out in a large open yard for a short period to be filmed, the development of rickets had progressed to the stage when not only were most of the lambs in these two groups showing the characteristic walk, but obvious bony enlargement of the distal ends of the radii had occurred in many of them. Pl. 1, 3 provides an illustration of the apparent enlargement of the carpus of no. 4 (group 1).

Vitamin D, when given to the sheep of group 3, prevented the appearance of any clinical sign of rickets, except in no. 6, which in August showed slight lameness, but no bone enlargement. All the sheep in groups 2 and 5 remained very lively throughout.

Ca:P ratio. From 20 October 1948 until 7 July 1949 two sheep—nos. 5 and 10—which had previously been given the high-P, high-Ca ration and thus acted as normal controls, were put on to the basal ration without supplementation with either P or Ca, that is, they received a diet low in P but normal in Ca. Analysis showed that the diet contained 0.087 % P and 1.25 % Ca. The effect upon growth was pronounced. Though these two sheep had maintained their rate of growth up to October (264 days) at 0.14 and 0.15 lb./day, it fell to 0.086 and 0.062 lb./day during the 210 days following the removal of additional P and Ca from the ration. Even when allowance is made for the natural decline in the rate of gain in weight with increasing age, this sharp fall may be fairly ascribed to the reduction in appetite due to the low-P diet. On 20 October 1948 the blood inorganic-P values for the two sheep were 6.7 and 6.6 mg./100 ml. By 21 April they had gradually fallen to 2.9 and 2.1 mg./100 ml., respectively, and were maintained at about this level until July. Total serum calcium remained at a normal level. In spite of this considerable fall in blood phosphate and the maintenance of so low a level for several months, associated with the exhaustion of reserves of vitamin D and its very low content in the ration, no evidence of rickets was observed from clinical and radiographic examination. This, indeed, is similar to Stewart's (1934-5) finding, and may mean that though the very wide Ca:P ratio of my experimental diet (29.6:1) made the diet strongly rachitogenic for sheep, it lost most of its rachitogenicity when the ratio was reduced to 14:1 by removal of the Ca supplement, even though the low P content of 0.08 % was unaltered.

Deaths of sheep during experiment. Three sheep died during the course of the experiment, and a fourth was slaughtered following infection of a rib-junction biopsy wound. With the first two deaths, following post-mortem examination and subsequent bacteriological and serological tests, the cause of death was found to be enterotoxaemia due to *Clostridium welchii* type D. These two cases occurred close together, one at the end of a balance trial on 17 June 1948 in no. 3, a low-P, high-Ca sheep, and the other 10 days later, in no. 19, a sheep from group 1 also on low-P, high-Ca diet. Both sheep died suddenly without showing any premonitory signs, as indeed is usually the way with this disease. Homologous anti-serum was at once obtained and all sheep were given the appropriate dose. The third death (no. 11, a high-P, high-Ca sheep) occurred on 22 December 1948 and was due to pneumonia. This animal had on several occasions gone off its food for short periods and once had to be withdrawn from a balance trial for this reason. A ruminal cannula had been inserted under general anaesthesia on 18 December and the animal was a very long time in regaining consciousness. Apparently it was these conditions of shock and lowered resistance allied to a weakness of constitution that resulted in the rapid development of pneumonia.

DISCUSSION

The basal diet used in this study provided a lower intake of phosphorus (0.3 g. daily) than that used by other workers studying the phosphorus metabolism of sheep; the diet used by Martin & Pierce (1934) gave 0.6 g., that used by Stewart (1934-5) gave over 1.0 g., and that of Du Toit *et al.* (1930) gave 0.47 g. per sheep. The vitamin D reserves of the lambs in the present work were not known but they would certainly be reduced during the 2 months pre-experimental period. The ration itself contained no more than 0.025 i.u. of vitamin D and thus would not supply what is believed to be the daily requirement of any of the sheep, and the greatest deficiency would be in those on the low-P diet, whose total food intake was so low.

Though the above factors were no doubt important in helping to make the diet so strongly rachitogenic, the main cause appeared to be the wide Ca:P ratio of 29.6:1, effected by high supplementation with calcium carbonate. When this was omitted, bringing the Ca:P ratio to 14:1, rickets did not occur in two of the sheep in spite of hypophosphataemia lasting several months. If the mechanism of rickets production with a wide Ca:P ratio is through the precipitation of phosphorus as insoluble calcium phosphate from an excess of Ca ions, then it must be presumed that there was enough unprecipitated phosphate when the dietary ratio of Ca:P was 14:1 to supply the minimum needs of sheep aged 20 months. This indication of the dependence of ruminants upon the Ca:P ratio of the feed is rather in contrast to the earlier South African work which emphasized the importance of intake levels rather than of the Ca:P ratio. In fact Theiler (1934) went so far as to say that his experiments had shown that the Ca:P ratio found to be so important in the aetiology of rickets in rats did not apply to ruminants, whose requirements for Ca are much lower than for P.

Massive doses of vitamin D₂ were markedly antirachitic and it is suggested that this may result in part from an increased turnover of phosphate from soft tissue for use in mineralizing bones, and partly from a reduction in the rate at which phosphate is excreted which would tend to conserve the available dietary phosphate. A subsequent communication (Ewer, 1951) deals with these points more fully.

SUMMARY

1. A study was made of the effect of phosphorus, calcium and vitamin D₂ on the aetiology of rickets experimentally produced in Welsh lambs.
2. A diet containing a minimum quantity of vitamin D (0.025 i.u./g.) and P (0.3 g.) daily but supplemented with Ca was found to be strongly rachitogenic.
3. Large doses of vitamin D₂ given orally (25 mg. calciferol in 10 ml. arachis oil) prevented the occurrence of rickets but had no effect upon growth. The protective action lasted approximately 2 months.
4. A similar dose of vitamin D₂ was effective in temporarily curing rickets in a sheep kept on the low-P, high-Ca diet.
5. The addition of disodium phosphate to bring the intake of P to 1.9 g./day prevented rickets and enabled the lambs to grow well.
6. Transferring two normal sheep from a high-P, high-Ca diet to the low-P diet



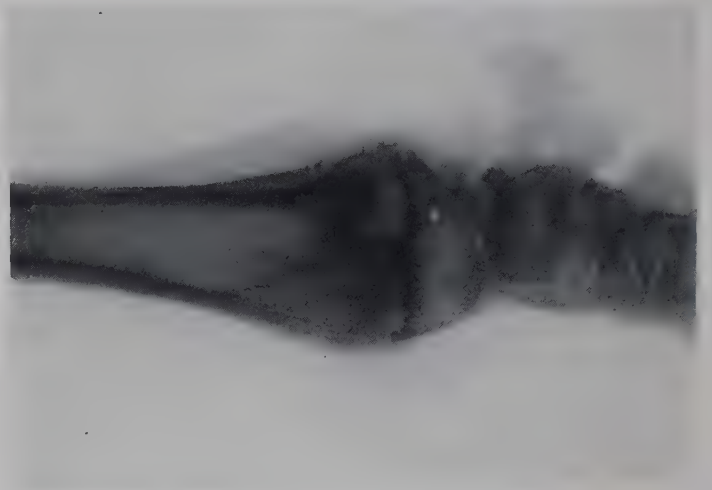
3



c



b



b



a

1



a

unsupplemented with Ca led to a fairly severe hypophosphataemia but no rachitic lesion.

7. The importance of a wide Ca:P ratio in the causation of rickets in sheep is emphasized. The low demand for vitamin D in sheep over 1½ years old when phosphorus intake is sufficiently high is also stressed.

The kindness of Miss E. Eden of the Dunn Nutritional Laboratory in undertaking the vitamin A analyses, and of Mr A. L. Bacharach and Dr W. F. J. Cuthbertson of Glaxo Laboratories Ltd. in undertaking the vitamin D assay, is most gratefully acknowledged.

EXPLANATION OF PLATE

1. Radiographs of sheep nos. 4 and 1. (a) Sheep no. 4 (basal diet only), active rickets. (b) Sheep no. 1 (basal diet with vitamin D), normal, 150 days after commencement of experiment.
2. Radiographs of sheep no. 17 (basal ration throughout, vitamin D given on 1 August 1948. (a) Rickets, 27 July 1948. (b) Normal, 9 September 1948. (c) Rickets again developing, 21 April 1949.
3. The swollen carpus of sheep no. 4 (basal diet), typical of well-developed rickets in lambs.

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Rickets in Sheep

2. Measurement of Phosphorus Absorption

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A previous paper (Ewer, 1951) described the production of rickets in lambs by means of a diet low in vitamin D and phosphorus and high in calcium, and the clinical effect of supplementation with vitamin D and phosphorus. Quantitative measurement of the P retention by these sheep forms the subject of the present communication. The value of balance trials in helping to establish quantitatively the absorption of various constituents of an animal's diet is well established. In rickets in the rat, dog and man, Ca and P metabolism has been investigated in this way by a number of workers and, in a study of Ca metabolism in sheep, Franklin (1934-5) also made use of the method. Bethke, Kick & Wilder (1932) reviewed the earlier work of McCollum, Simmonds, Parsons, Shipley & Park (1920-1) and of Sherman & Pappenheimer (1920-1) and found that the optimum Ca:P ratio for the rat was between 1.0 and 2.0.

It has been shown that the digestibility of many of the major food constituents in a sheep's diet, including dry matter, can be affected by the addition of such things as crude fibre, readily available carbohydrate and nitrogenous compounds (Swift, Thacker, Black, Bratzler & James, 1947), and that the ash from lucerne hay can increase the digestibility of roughage from ground maize-cobs (Burroughs, Gerlaugh & Bethke, 1950). Observations were therefore made upon the dry-matter digestibility

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of the basal diet following single massive dosage with vitamin D₂ and alteration of the Ca:P ratio by additional phosphate. It was thought desirable also to investigate the P metabolism of affected and normal sheep, while they were kept on a rachitogenic diet, and to observe the effect of supplementation by vitamin D.

EXPERIMENTAL

Sheep. The sheep were placed in the usual metal metabolism crate or in a specially constructed raised wooden pen, through the floor of which a hole was bored to allow the passage of a rubber tube connected to a wide-mouth enamel funnel, held in place on the belly of the sheep by a wide rubber band. Faeces were collected in rubberized canvas bags held in position by leather harness. Water was always available to the sheep from troughs clipped to the side of the pen. Analyses indicated that the tap water contained a negligible amount of P, although Ca was present to the extent of 5.68 mg./100 ml. In view of the small quantity of either mineral likely to be added from this source to the total daily intake, tap water was used throughout.

The nervous temperament of the young Welsh sheep used made it necessary to have rather long pre-experimental periods to allow them to become accustomed to pens and harness, as indicated by their general behaviour and the constancy of the food intake.

Food. A representative sample of the food after it had been mixed each morning, and the whole of the residues of the preceding day's feed were taken. It was subsequently found that the maximum variation in the P content of the food residues of any particular sheep from day to day was within 0.1 mg. of the mean value. The residues were therefore bulked from each sheep, and after careful mixing, a sample was taken for dry-matter and P determination. *Dry matter* was estimated by drying in an electric oven at 100° to constant weight. *Phosphorus* was estimated by taking duplicate 1 g. samples of the dried material after grinding, and ashing them with excess 10 % Mg(NO₃)₂ in a muffle furnace at 540°. The ash was dissolved in 6N-HCl, made up to volume and an appropriate portion taken for P determination according to Gomori's (1942) modification of Fiske & Subbarow's (1925) method.

Urine. A crystal of thymol was placed in the urine pots and each 24 hr. collection measured. After mixing, a 250 ml. sample was taken for analysis. Acid-soluble P was determined on a 10 % trichloroacetic-acid filtrate and total P by wet digestion with 60 % perchloric acid and hydrogen peroxide.

Faeces. The bags were removed at 24 hr. intervals and the faeces weighed, dried at 100° to constant weight and ground. It was found that the daily P variation in the faeces of any particular sheep compared to a pooled sample was small, so that in most trials the dried faeces of each sheep for the whole trial were ground together and duplicate samples taken for ashing. The P content was determined as for the food.

Exp. 1. Six sheep were used, three from a group being given a rachitogenic, low-P diet and three from a group given the same diet but having had a massive dose of vitamin D₂ by mouth 12 days before. Further details of food and management of the sheep used in this and subsequent experiments will be found in the preceding paper (Ewer, 1951).

Exp. 2. Three sheep that had been receiving the low-P diet and were showing well-developed rachitic lesions, as indicated by X-ray examination and blood inorganic-P values of 1.8 or 1.9 mg./100 ml., were compared with three sheep from a high-P group that were clinically normal and had blood inorganic-P levels of between 5.4 and 6.1 mg./100 ml.

Exp. 3. This experiment only lasted 6 days, since the sheep were being used primarily for the investigation of the excretion of ^{32}P . Four rachitic sheep and two normal (high-P) sheep were used. At the conclusion of the pre-experimental period, two of the low-P sheep and one of the high-P animals were taken at random and a large dose of vitamin D was given subcutaneously. The following day, ^{32}P was given intravenously and the experimental period began.

Exp. 4. Observations are reported on only one sheep which served as its own control and was being used in isotopic-P excretion studies. This animal, no. 4, had been rachitic for over 12 months as a result of being kept on the low-P, high-Ca diet. The amount of food offered was set at a level of 2030 g. daily (wet weight), and during the experiment food residues remained at a constant level.

RESULTS

Exp. 1. The results of the first experiment are summarized in Table 1. Although in no way conclusive, they suggest that the vitamin D-treated sheep may have been more nearly in P balance than the others. Closer examination of the two exceptions revealed that sheep no. 2 had the least rickets according to radiographic evidence, and that sheep no. 15 was losing weight at the time of the experiment and not long afterwards showed a moderate degree of rickets.

Table 1. *Exp. 1. Digestibility of dry matter and mean daily retention of phosphorus by the sheep over 10 days*

Sheep no.	Diet	Dry matter			Retention of P (g.)
		Intake (g.)	Output (g.)	Digestibility (%)	
2	Low-P (all sheep rachitic)	439.2	90.0	79.5	+0.042
3*		406.7	96.1	76.4	-0.093
18		(7 days only)	(7 days only)		
		505.6	92.9	77.7	-0.050
1	Low-P with vitamin D (sheep not rachitic)	116.2	20.2	82.6	+0.009
6		255.7	35.3	86.2	+0.053
15		117.9	21.8	81.5	-0.085

* Sheep no. 3, on the 8th day, left nearly half its food and began suddenly to pass watery faeces. On the morning of the 9th day it was found dead. A post-mortem examination indicated death from enterotoxaemia due to *Clostridium welchii* type D, which was confirmed by cultural and toxin-antitoxin tests.

The higher digestibility coefficient for the vitamin D-treated sheep should be considered in relation to their very much lower food intake. In the 30-day period before the balance trial started the group from which these sheep were taken con-

sistently showed a food consumption 21 % lower than that of the unsupplemented low-P group.

Exp. 2. The 3 weeks' preliminary period established the maximum dietary levels at 1200 g./sheep/day for the rachitic sheep and at 2500 g./sheep/day for the normal animals. Table 2 summarizes the data concerning P absorption and dry-matter digestibility.

Table 2. Exp. 2. Digestibility of dry matter and mean daily retention of phosphorus by the sheep

Sheep no.	Diet	Dry matter			Retention of P (g.)
		Intake (g.)	Output (g.)	Digestibility (%)	
4 } 13 } 20 }	Low-P (all sheep rachitic)	411.4	94.9	76.9	-0.089
		409.0	95.5	76.6	-0.075
		421.2	91.0	78.4	+0.011
5 } 10 } 12 }	High-P	879.7	153.0	82.4	+0.501
		927.9	175.0	81.1	+0.547
		804.2	145.3	81.9	+0.621

In view of the clinical condition of the low-P sheep it was not surprising to find that two of them were in negative P balance. The mean daily intake of P was 0.321 g. and was similar to that attained by the low-P sheep in Exp. 1, namely 0.345 g. It probably represents the minimum intake for maintenance on this particular diet.

It will be noted that an increase occurred in the digestibility of the dry matter in the P-supplemented sheep, in spite of the fact that the intake of dry matter was so much higher in this group.

Exp. 3. The results for the short 6-day trial, where sheep nos. 6, 13 and 12 were given vitamin D at the dose rate of 20,000 i.u./kg. 24 hr. before the experiment began, are given in Table 3.

Table 3. Exp. 3. Digestibility of dry matter and mean daily retention of phosphorus by the sheep over 6 days

Sheep no.	Diet	Dry matter			Retention of P (g.)
		Intake (g.)	Output (g.)	Digestibility (%)	
1 } 2 }	Low-P (sheep rachitic)	265.5	46.0	82.7	+0.048
		277.5	49.6	82.2	+0.064
14	High-P (sheep normal)	405.7	68.7	83.0	-0.164
6 } 13 }	Low-P (sheep rachitic)	368.6	68.7	81.4	+0.137
		381.3	73.8	80.6	+0.084
12	High-P (sheep normal); all these three received vitamin D ₂	476.0	88.1	81.4	-0.387

The low intakes of sheep nos. 1 and 2 were due to a reduction in appetite which followed injection of the ³²P, although the dose was well within what are thought to be safe limits, and the sheep lost weight during the trial. This reduced intake was probably the reason for the raised digestibility of the dry matter for these sheep, since in this they were comparable to the sub-maintenance sheep in Exp. 1.

The negative balances of both high-P sheep were surprising and may be related to the fact that their food consumption was only half that of previously studied high-P sheep. The rachitic sheep given vitamin D (nos. 6 and 13) retained more P than the unsupplemented sheep (nos. 1 and 2), but since the food intake of nos. 6 and 13 was so much higher than that of the other two, it cannot be stated whether this higher retention was due to these sheep being less subject to the radiotoxic effect of ^{32}P , or to a direct effect of the vitamin upon appetite. The evidence from sheep no. 4 in Exp. 4 supports the latter possibility.

Exp. 4. After the first 10-day experimental period, the one sheep used in this experiment was left just over a month before the second period. At an interval of 12 hr. before the second period began 1,000,000 i.u. vitamin D_2 were given subcutaneously. The results are summarized in Table 4.

Table 4. *Exp. 4. Digestibility of dry matter and retention of phosphorus in sheep no. 4 (low-phosphorus, high-calcium, rachitic)*

	Before administration of vitamin D_2				After administration of vitamin D_2			
	Intake (g.)	Output (g.)	Re- tention (g.)	Digesti- bility (%)	Intake (g.)	Output (g.)	Re- tention (g.)	Digesti- bility (%)
P	0.297	0.264	0.033	—	0.291	0.242	0.049	—
Dry matter	356.0	75.7	—	78.7	320.2	63.96	—	80.0

The evidence from this sheep supports the finding in the earlier experiments that the effect of a massive dose of vitamin D_2 is to increase both P retention and the apparent digestibility of the dry matter.

DISCUSSION

On the low level of P intake in these experiments, the total urinary P was only 1–2 % of the quantity in the faeces. There was no alteration in the urinary P level following vitamin D_2 administration. In the last experiment, for instance, the mean daily P value in the urine both before and after administration of vitamin D, was 0.004 mg./100 ml. Watson (1933) pointed out that the threshold value of P is high in sheep, and it was therefore not surprising to find that it was generally only in the sheep with a high P intake that any measurable quantity of acid-soluble P was found. The effect of the vitamin on the concentration of P in the faeces varied somewhat with different sheep but was not pronounced, e.g. in sheep no. 4, used in the last experiment, the effect was to increase the mean daily excretion by 15 mg./100 g.

It appears that the increase in both the P retention and digestibility of dry matter which followed treatment with vitamin D was achieved through a lowering of the total amount excreted relative to the intake. In view of the finding of Du Toit, Malan & Rossouw (1930) that digestibility is unaltered by the level of P intake, it was particularly interesting to find that the digestibility of dry matter for the rachitic sheep in the present work was raised when phosphate was added to the diet. The effect of the phosphate deficiency was not only to restrict the appetite and so the consumption of

dry matter, but also the capacity to digest it. This recalls the work of Riddell, Hughes & Fitch (1933) who found an increased metabolic rate in cows suffering from aphosphorosis and suggested that this might be the explanation of the lowered efficiency of food utilization originally observed in cattle by Theiler, Green & Du Toit (1924).

The relative reduction in faecal dry matter which appears to follow massive dosing with vitamin D may in part be due to decrease in food residues following more efficient bacterial and protozoal breakdown in the rumen, wherein a more favourable nutrient medium may be provided by an increased rate of P turnover through the saliva. It may also in part be due to a reduction in the number of dead micro-organisms which, at least in non-ruminants, form the bulk of the dry matter of the faeces (Henry & Kon, 1939).

Investigations by Cohn & Greenberg (1939) in the rat and by Shimotori & Morgan (1943) in the dog lead to the conclusion that vitamin D supplementation in rachitic animals of these species does not cause an increase in the uptake of P from the gut. The higher P retention of the sheep treated with vitamin D in the present work may indicate that in ruminants either increased absorption or decreased 'endogenous' excretion into the gut, or both, may occur when deficient animals are given vitamin D. Subsequent preliminary work, using radioactive P (Ewer, 1952), indicated that the mechanism operating in the sheep is very complex.

SUMMARY

1. The results are reported of a small series of balance trials, designed to ascertain the effect of a single massive dose of vitamin D₂ on P retention and on the digestibility of the dry matter eaten by normal and rachitic sheep.
2. Most of the rachitic sheep that maintained their weight were in negative P balance.
3. The effect of giving one large dose of vitamin D₂ was to increase P retention, and apparently the effect was sufficient in some instances to make negative balances positive.
4. The utilization of feed in terms of dry matter was increased approximately to the same degree by both high-P supplementation and dosing with vitamin D.

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Tryptophan Deficiency and Requirements in the Adult Rat

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Many investigations have been made in recent years on the nutritive value of protein hydrolysates and amino-acid mixtures. Nevertheless, it is doubtful whether these are complete substitutes for whole protein. The result of the first experiment reported here suggested that acid-hydrolysed casein supplemented with tryptophan was inadequate both for the maintenance of the adult female rat and for promoting recovery of animals suffering from severe tryptophan deficiency. However, the results of later experiments indicated that the supplemented hydrolysate was only slightly inferior in nutritive value to whole casein for adult, growing and tryptophan-deficient rats. In these experiments it was found possible to maintain adult rats at a constant, if not maximal, weight level on the acid-hydrolysed casein when it was supplemented with tryptophan, and an attempt was made to determine the maintenance requirement of the adult rat for tryptophan for comparison with values reported for the growing rat. At the same time the effects of prolonged tryptophan deficiency were studied, since it was thought that by using only adult animals it would be possible to distinguish between any specific effects that might be caused by this deficiency and those that were secondary to the general stunting.

EXPERIMENTAL AND RESULTS

General

Management of rats. The experiments were performed on hooded rats of the Lister strain. Litter-mates were used and divided into groups, as far as possible similar with regard to age, sex and weight. They were kept in individual cages with mesh bottoms placed in a tray containing sawdust (Exp. 1), or lined with blotting paper to absorb the urine (Exps. 2-4). This made it possible to collect the food that had been scattered and to determine the food intake. For the most part the faeces fell through the mesh; no extra precautions were taken against coprophagy.

Diets. In all the experiments the animals were fed *ad lib*. The percentage composition of the basal diets was: casein (whole or hydrolysed) 14.7, cystine 0.3, starch 62, hardened arachis oil 15, lard 3 and salts 5. The salt mixture had the following composition: NaCl 51.9, MgSO₄·7H₂O 164, NaH₂PO₄ 104.1, K₂HPO₄ 286.2, Ca(H₂PO₄)₂·H₂O 162,

Ca lactate 390, ferric citrate 35.4 parts. The dry constituents of the diet were well mixed, an equal weight of water was incorporated, and the mixture then steamed until the starch was thoroughly cooked. The diet, when cool, set to a cake of cheese-like consistency.

The acid-hydrolysed casein was prepared by the method of Jackson (1927). The vitamin supplements, which differed slightly, are recorded under the description of the individual experiments. One batch of the food-yeast extract contained in $\mu\text{g./g.}$ extract: riboflavin 300, nicotinic acid 2000, pantothenic acid 400, biotin 0.4 and pyridoxin 100. It had a low amino-acid content and gave a negative glyoxylic acid test for tryptophan.

Exp. 1. Effect on body-weight of casein and its hydrolysates

Arrangement of experiment

Twelve young adult female rats from four litters between 129 and 194 days old were divided into three groups. As the protein fraction, the diets contained whole (unhydrolysed) casein (U.C.), acid-hydrolysed casein (A.H.C.), acid-hydrolysed casein with 0.2 g. L-tryptophan/100 g. diet (A.H.C.T.) or a papain and pancreatin digest of casein (E.H.C.). They were given to the three groups according to the details given in Table 1. Vitamins were supplied to each rat as follows: 1 ml. yeast extract (equivalent to 500 mg. yeast) and 15 $\mu\text{g.}$ aneurin hydrochloride daily, three drops cod-liver oil five times a week and three drops of α -tocopherol solution twice a week (equivalent to 8 mg./week).

Table 1. *Average change in body-weight when groups of four adult rats received diets containing whole casein (U.C.), tryptophan-free acid-hydrolysed casein (A.H.C.), A.H.C. supplemented with 0.2% L-tryptophan (A.H.C.T.) and an enzymic digest of casein (E.H.C.)*

Group A (Average initial weight 250 g.)				Group B (Average initial weight 235 g.)				Group C (Average initial weight 235 g.)			
Length of period (days)	Diet given	Average change in weight (g.)	Length of period (days)	Diet given	Average change in weight (g.)	Length of period (days)	Diet given	Average change in weight (g.)	Length of period (days)	Diet given	Average change in weight (g.)
43	U.C.	-22	43	A.H.C.T.	-41	63	A.H.C.	-120	43	U.C.	+3
43	A.H.C.T.	-47	64	U.C.	+21.5	23	A.H.C.T.	+3	47	A.H.C.T. with tryptophan increased to 0.4%	+6
47	A.H.C.T. with tryptophan increased to 0.4%	+5	46	E.H.C.	-12	10	A.H.C.T. with tryptophan increased to 0.4%	+65	20	U.C.	+19.5

Results

The animals of group A on transference from the stock diet to diet U.C. showed a weight loss that was least with the two smaller animals. After 3-4 weeks the weights of all the animals were similar (220, 228, 227 and 236 g.), regardless of their initial weights (225, 279, 236 and 261 g.). Little change occurred during the last 2 weeks of the period, but when the animals were transferred to diet A.H.C.T. there was

a further loss of weight and inhibition of oestrous cycles. Doubling the tryptophan content of the diet prevented further loss, but in only one of the four animals did it cause an increase (15 g.) of more than 5 g. in 47 days. However, during period 4 when the rats were returned to diet U.C. (Table 1, Fig. 1) a slow increase in weight occurred in all the animals, and normal oestrous cycles were restored. The weight curve of one of the rats is shown in Fig. 1.

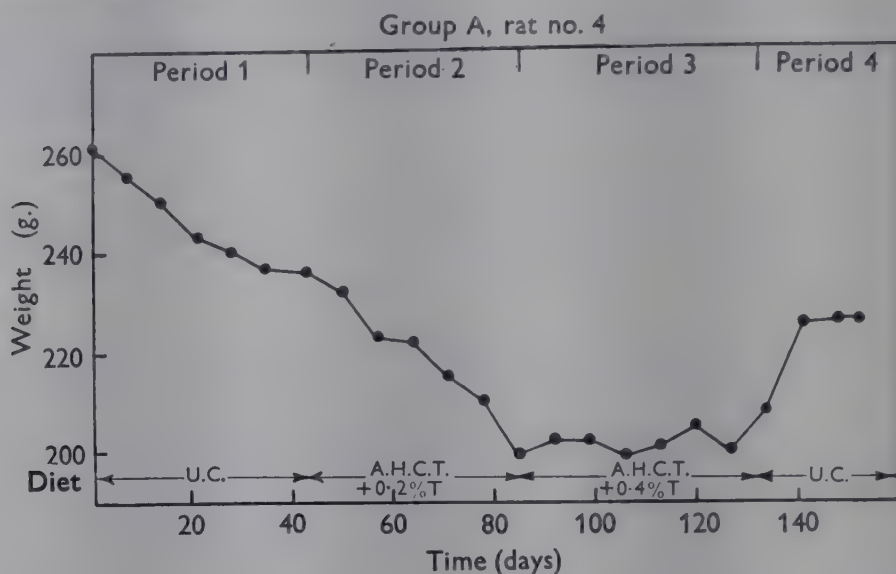


Fig. 1. Exp. 1. Failure of diet A.H.C.T. to maintain adult female rats at their original weight level. For details of diets see p. 307.

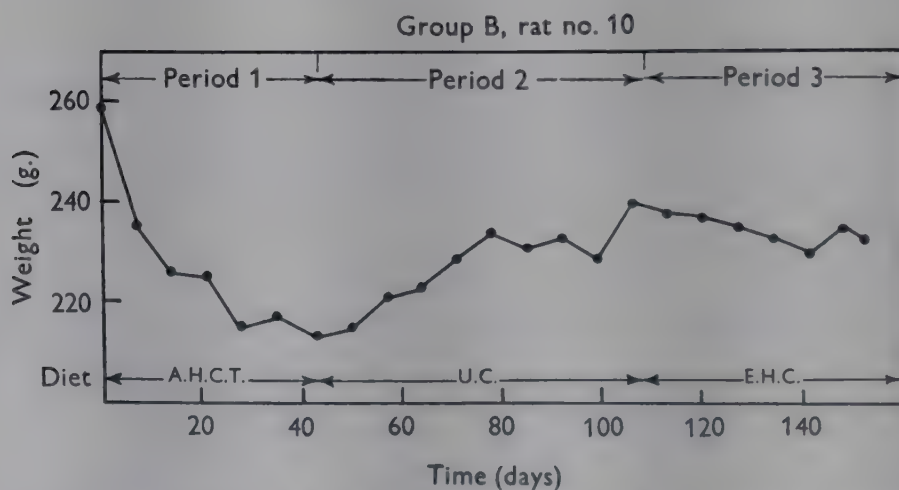


Fig. 2. Exp. 1. Failure of diet A.H.C.T. to maintain adult female rats at the level achieved with diet U.C. For details of diets see p. 307.

Similar results were obtained in group B. During period 1, when the rats of this group were given diet A.H.C.T., considerable weight loss occurred which again was greater in the heavier animals. During period 2 when they received diet U.C., they all made slow increases in weight, although a further loss of weight occurred slowly during period 3 when diet E.H.C. was given. The weight curve of one of these rats is shown in Fig. 2.

The animals of group C received the tryptophan-deficient diet (A.H.C.) for 63 days, during which they lost 46.7, 56.0, 49.6 and 44.0 % of their initial body-weights respectively. During the last few days of this period the rate of loss increased and the

condition of the animals became critical. In an attempt to restore them, 0.2 % tryptophan was added to the diet, but the overall weight changes of 0, 15, 8 and -1 g. in 23 days were slight, and there were considerable day-to-day fluctuations. For the second rat the weight curve suggested that slow recovery was initiated by the inclusion of 0.2 % tryptophan in the diet, but during the following 10 days when the amount was increased to 0.4 %, there was a further gain in weight of only 2 g. The weight changes for the other three animals after increasing the tryptophan were

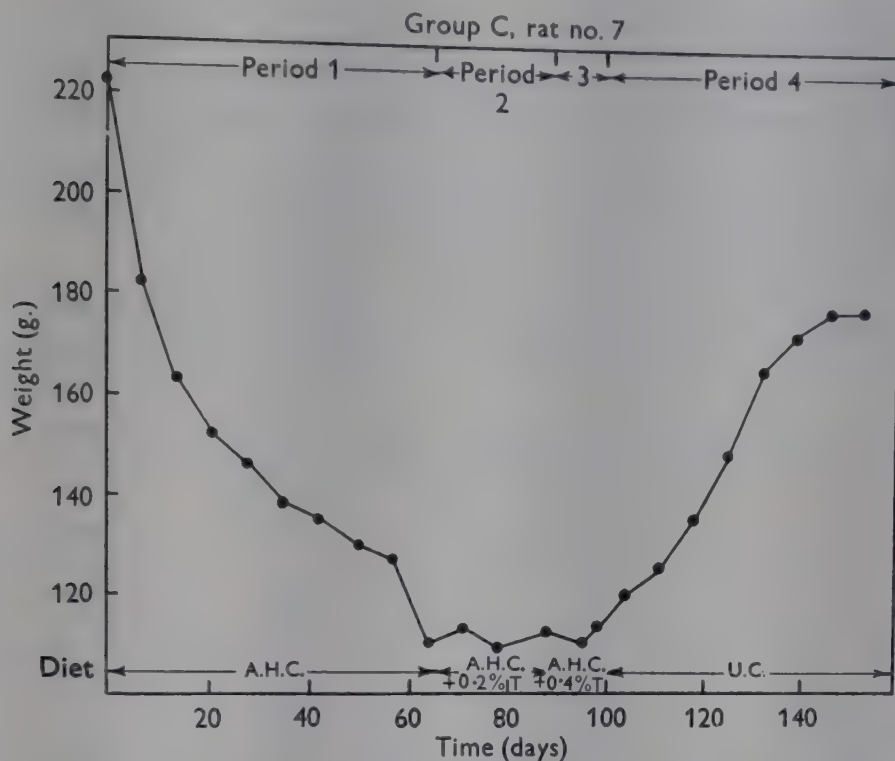


Fig. 3. Exp. 1. Failure of adult tryptophan-deficient rats to recover on the addition of tryptophan to the diet. For details of diets see p. 307.

+7, +1 and +5 g. giving total weight gains of 7, 17, 9 and 4 g. for the 33 days during which tryptophan was given. However, when the rats were transferred to diet U.C. they made immediate and progressive weight gains, and oestrous cycles were restored in from 25 to 40 days. The weight curve of one of these rats is shown in Fig. 3.

The addition of tryptophan to the diet of these animals stopped the steep decline in weight, and was accompanied by an increase in food consumption. This could not be measured accurately owing to some scattering of the food among the sawdust but was about 50 g./rat during the last week of the deficiency period (29 Cal./100 g. body-weight/day) and 77 g. for the 1st week after the addition of tryptophan (44 Cal./100 g. body-weight/day). Later, when the tryptophan content of the food was increased, the food intake appeared to be slightly reduced and the feeding of whole casein did not seem to increase the intake above that seen with diet A.H.C.T. until after the animals had made an appreciable weight gain.

Exp. 2. Effect on body-weight of young rats of a diet containing acid-hydrolysed casein supplemented with tryptophan

The failure of diet A.H.C.T. to maintain adult rats and to enable those suffering from tryptophan deficiency to recover was particularly surprising in view of the known adequacy of such diets for growth in young animals (Jackson, 1927; Berg & Rose, 1929). In the circumstances it was decided to investigate the ability of diet A.H.C.T. to support the growth of young rats of the same strain.

Arrangement of experiment

Groups of four young rats aged 30–33 days and weighing between 62 and 95 g. were used. The composition of the diets was similar to that in the previous experiment except that, in addition to the vitamin supplements given in Exp. 1, each animal was supplied daily with pyridoxin 30 μ g., riboflavin 50 μ g., calcium pantothenate 100 μ g., nicotinic acid 1 mg., inositol 1 mg. and choline chloride 6 mg.

The food residues were collected on blotting paper which lined the trays and absorbed the urine. They were separated from the faeces and weighed daily.

Table 2. *The calorie intake and change in body-weight of young rats given diets A.H.C.T.* and U.C.**

	Diet A.H.C.T.					Diet U.C.					
Rat no.	...	21	22	23	24	Average	25	26	27	28	Average
Initial weight (g.)		93	62	70	72	74	85	73	79	82	80
Final weight after 8 weeks (g.)		169	128	178	182	164	198	174	199	253	206
Weight increase: (g.)		76	66	108	110	90	113	101	120	171	126
	(%)	77	106	154	153	122	138	138	151	208	159
Total calorie intake (Cal.)		3485	2633	3516	3241	3219	3673	3424	3738	4329	3791

* See Table 1.

Results

The results are shown in Table 2. Satisfactory growth occurred on both diets U.C. and A.H.C.T., and statistical analysis of the results showed that there was no significant difference in the rates of growth of the two groups. The average total gain in weight with its standard error of an animal in 8 weeks on diet U.C. was 126 ± 16 g. and on diet A.H.C.T. it was 90 ± 11 g., with $t = 1.49$; the probability that the difference was significant was $P > 0.1$. In each group there was a correlation between the weight increase and the calorific value of the food intake (correlation coefficient $r = +0.77$).

The failure of the rats of Exp. 1 to recover when fed diet A.H.C.T. remained unexplained.

Exp. 3. Further investigations of observations made in Exp. 1

Arrangement of experiment

In an attempt to investigate more fully the observations made in Exp. 1, twelve adult female rats were made tryptophan-deficient by diet A.H.C. The vitamin supplements were similar to those of Exp. 2 except that the choline chloride was increased

from 6 to 12 mg./rat/day. After 49 days when the animals had lost an average of 35.9 % of their initial weight, they were divided into groups of four which were then given diets U.C., A.H.C.T. and E.H.C. respectively.

Results

The results are shown in Fig. 4. Recovery occurred in all three groups of animals regardless of the nature of the diet. Five days after changing from the deficient diet, when all the animals were rapidly gaining in weight, two from each group were returned to the deficient diet and their subsequent treatment is described under Exp. 4. The other two in each group were allowed to continue on their respective recovery diets for 3 weeks or more to ensure that their recovery was continuous.

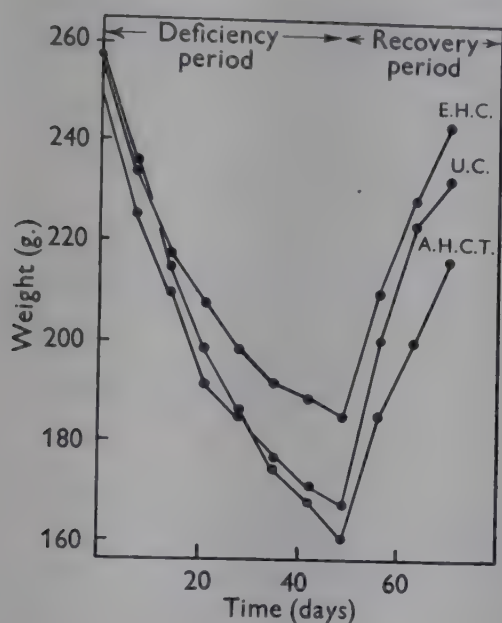


Fig. 4. Exp. 3. Recovery of weight in adult tryptophan-deficient rats when fed diets U.C., E.H.C. and A.H.C.T. For details of diets see p. 307.

Exp. 4. Determination of the tryptophan requirement of the adult rat

Arrangement of experiment

At the conclusion of Exp. 3 the animals, together with a further group of four rats which had been treated similarly, were used to determine the tryptophan requirement of the adult rat. In addition a further four rats (group 1) received the tryptophan-deficient diet until they were moribund. The others were divided into groups of four, two of which had almost completely recovered from the deficiency, the other two having been depleted for the second time. These groups (2-5) all received diet A.H.C. supplemented with 0.01, 0.05, 0.1 and 0.2 % tryptophan respectively.

One of the rats of group 1 died from a respiratory infection after 41 days on the deficient diet and was not included in recording the results. The effects of total deprivation of tryptophan were studied in the three remaining rats and also, so far as gross results were concerned, in the four deficient rats (group C) of Exp. 1. Red-corpuscle counts and haemoglobin values were determined and the results are recorded in Table 3. Included for comparison are the results obtained for two animals which,

Table 3. *Haemoglobin estimations and red-corpuscle counts on groups of adult rats given diets U.C.*, A.H.C.*, and A.H.C.* supplemented with different amounts of tryptophan*

Group no.	Rat no.	Diet	Haemoglobin (g./100 ml.)	Red-corpuscle count (millions/cu.mm.)
Control	C3	Stock	14.6	9.14
	C4		14.6	9.50
	C7		15.6	8.87
	C9		14.1	8.56
	Average		14.7	9.02
6	118	U.C.	14.3	9.53
	119		14.1	8.35
	Average		14.2	8.99
5	122	A.H.C. with 0.2 % tryptophan	13.4	8.12
	135		14.1	8.86
	Average		13.8	8.49
4	120	A.H.C. with 0.1 % tryptophan	13.8	8.96
	127†		13.2	8.74
	Average		13.5	8.85
3	128	A.H.C. with 0.05 % tryptophan	13.7	8.61
	136		14.7	10.11
	Average		14.2	9.36
2	123†	A.H.C. with 0.01 % tryptophan	6.6	3.96
	126†		11.5	7.29
	129		12.3	8.46
	132		13.1	8.65
	Average (of 129 and 132 only)		12.7	8.55
1	113	A.H.C. unsupplemented	7.7	4.61
	115		10.4	—
	116		10.0	4.57
	Average		9.4	4.59

* See Table 1.

† Small blood clots present.

immediately after the deficiency period, increased in weight on diet U.C. Oestrous cycles were studied by the vaginal smear method.

In the latter part of Exp. 4 normal supplies of the acid-hydrolysed casein became inadequate, and for a short period it was necessary to mix the ordinary preparation with equal quantities of one containing some calcium sulphate, or to use the latter preparation alone. Changes in the hydrolysate caused minor weight changes but did not interfere with the main result.

Results

The effects of tryptophan deficiency. The four rats of Exp. 1 received the deficient diet for 63 days and lost between 44 and 56 % of their initial body-weight. Their condition was then critical. The three rats of group 1 (Exp. 4) were killed after 106–112 days on the deficient diet, when they were on the point of death and had lost between 51.6 and 58.1 % of their original weight.

In addition to the usual features associated with nutritional deficiency, e.g. emaciation, staring coat, anorexia and permanent dioestrus, there was necrosis of the tail and staining of the wrists, paws and nose with reddish material. Although oestrus was inhibited 10–25 days after the period of tryptophan-deficiency began, the four animals of Exp. 1 showed normal cycles after recovery on a diet containing whole casein, and two of them subsequently bore young, which, however, were either born dead or died shortly after birth.

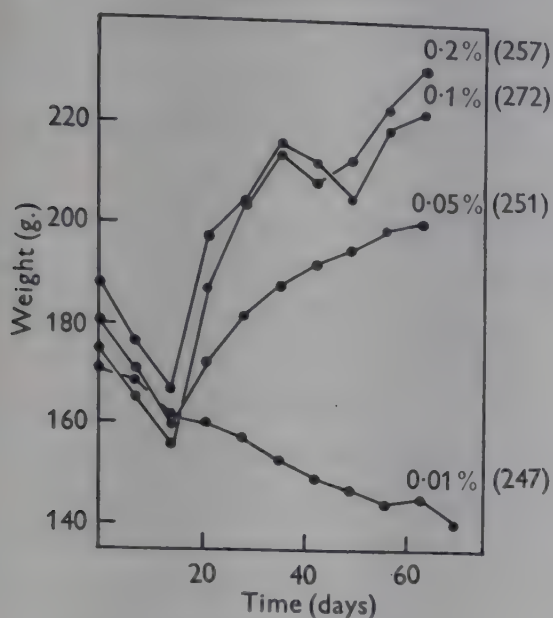


Fig. 5

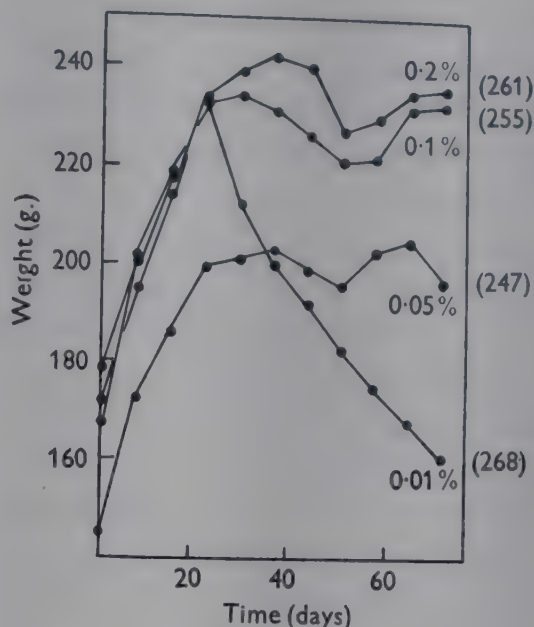


Fig. 6

Fig. 5. Exp. 4. Effect of inclusion of tryptophan at various levels in the diet of tryptophan-deficient rats. The figures in parentheses show the average initial weights of the rats in g.

Fig. 6. Exp. 4. Effect of feeding tryptophan at various levels to rats that had partly recovered from a period of tryptophan deficiency. The figures in parentheses show the average initial weights of the rats in g.

Group 2, given 0.01 % tryptophan. The animals of this group showed a progressive loss of weight which was more rapid in the two animals that had recovered from the initial deficiency. Not only did 0.01 % tryptophan fail to maintain the animals at any weight level, but in three of the four animals the rate of loss was not appreciably less than when tryptophan was altogether lacking. All the animals of this group showed complete inhibition of oestrus, and symptoms of deficiency similar to those described in the rats totally deprived of tryptophan.

Group 3, given 0.05 % tryptophan. The rats that had nearly recovered from the deficiency before being given this diet did not complete their recovery, but maintained themselves at a weight level considerably below their initial values for 7 and 11 weeks, respectively, until they were killed. The two deficient animals showed a slow weight increase on this level of tryptophan, followed by maintenance at a level of 190–210 g., similar to that of the other two animals of the group. This level seemed to be independent of the initial weight of the animals, which varied between 231 and 271 g. All were in fairly good condition but noticeably thin. Oestrous cycles returned but there was a tendency to periods of continuous cornification of the vaginal mucosa, or prolonged intervals between the cycles. Some of the results are shown in Figs. 5 and 6.

Group 4, given 0.1 % tryptophan. The animals of this group that had already recovered much of their lost weight, maintained themselves for the remaining 7-11 weeks on this level of tryptophan. The smaller animal slightly surpassed its initial weight (234 g.) and remained at this level, whereas the larger did not fully regain its initial weight (277 g.).

The rats that were transferred direct from the deficient to the 0.1 % tryptophan diet showed a steady increase in weight except for a short period during which the hydrolysate containing calcium sulphate was given. Here again the smaller animal nearly reached its initial weight but the heavier did not do so within the experimental period, although its weight was still increasing slowly when it was killed. All the animals appeared to be in good health, and oestrous cycles, inhibited during the course of the deficiency, were restored.

Group 5, given 0.2 % tryptophan. The results for this group did not differ materially from those of the 0.1 % tryptophan group. The smaller animals regained the whole of their lost weight, but recovery of the larger ones was incomplete. The health of all the animals appeared good and oestrous cycles were normal.

Blood analyses. The results of the blood analyses are shown in Table 3. There was pronounced anaemia in the three rats given the totally deficient diet. The haemoglobin level fell to 63.8 % of the value recorded for rats given the stock diet. Owing to clumping of the corpuscles in the blood of one of the rats on dilution it was impossible to make a red-corpuscle count. In the other two, however, the counts were 4.61 and 4.57 million/cu.mm. respectively, as compared with an average of 9.02 for the controls. The values for the two animals given diet U.C. during recovery from the deficiency were only slightly lower than those of the controls given the stock diet, and those of the animals that had recovered after supplementation of the deficient diet with 0.2 % tryptophan were comparable with them. Values as high as those seen in the latter group were found in the animals given diet A.H.C. supplemented with 0.1 and 0.05 % tryptophan.

A sample of the reddish material found on the nose, paws and feet of the deficient rats when washed off with water gave a faint-brown cloudy solution which did not give a positive benzidine test. Spectroscopic examination showed an intensification of the *D* line and a faint band in the green region suggesting that the pigment might be a porphyrin.

DISCUSSION

General effects of tryptophan deficiency

The general condition of the tryptophan-deficient rats was similar to that seen in other forms of protein deficiency except for the caudal necrosis which had not previously been reported in protein or amino-acid deficiencies. The weight loss, averaging 8.9 % in the 1st week and 2.0 % in the 15th week, was much greater than that reported by Neuberger & Webster (1945) in adult male rats suffering from lysine deficiency, but nevertheless the animals survived for periods up to 112 days.

The inhibition of oestrus caused by tryptophan deficiency cannot be considered specific, since deficiency of almost any essential nutrient has this effect. Albanese,

Randall & Holt (1943) found that foetal resorption occurred in female rats given a tryptophan-deficient diet immediately after mating, and Keller (1946) reported that young rats of both sexes when given a tryptophan-deficient diet for from 3 to 18 days became sterile and showed no signs of sexual activity for the remainder of the observation period of 90–150 days. His findings, however, are not supported by the work of Berg & Rohse (1947) nor by the results of Exp. 1, where recovery from extreme tryptophan deficiency led to return of oestrous cycles, two of the rats concerned subsequently bearing young.

The anaemia seen in the rats of Exp. 4 was more pronounced than that previously reported in tryptophan deficiency (Alcock, 1933; Hamada, 1936; Albanese, Holt, Kajdi & Frankston, 1943), but, since adult rats were used in the present work, comparison with results mostly obtained on immature animals is difficult. Tryptophan, however, is not alone among the amino-acids in being essential for the maintenance of normal haemoglobin values, and anaemia has also been reported in deficiencies of lysine, methionine, histidine, phenylalanine and isoleucine. It is extremely doubtful therefore whether tryptophan plays a specific role in blood formation, although suggestions of a direct stimulant action (Matsuoka & Nakao, 1931) have not been finally disproved.

The results of the analyses performed on rats that had recovered from the effects of tryptophan deficiency of 49 days' duration suggest that there was no permanent damage to the haemopoietic system. The rats that received 0.05 % tryptophan had haemoglobin values comparable with those of the rats that received 0.1 and 0.2 % tryptophan, although the 0.05 % group was being maintained at a lower weight level. The haemoglobin values and red-corpuscle counts of the rats receiving 0.01 % tryptophan were higher than those of the totally deficient rats, although the loss of body-weight was comparable, averaging 51.6 and 53.8 % respectively. This finding, which is in agreement with the observation of Hamada (1936) that in young rats given tryptophan at a level of 0.025 % the haemoglobin rose although the weight fell, suggests that small amounts of tryptophan may be used preferentially in the maintenance of haemoglobin levels.

The exact nature of the reddish brown material deposited on the nose and paws of the deficient rats was not determined. Krehl, Sarma, Teply & Elvehjem (1946) found a similar accumulation of porphyrin-like material in young rats fed a diet low in nicotinic acid and containing maize. This is of particular interest in view of the relationship now known to exist between tryptophan and nicotinic-acid metabolism (Krehl, Teply, Sarma & Elvehjem, 1945). Porphyrin deposition has also been reported in pantothenic-acid deficiency (Chick, Macrae & Worden, 1940; McElroy, Salomon, Figge & Cowgill, 1941).

The adequacy of supplemented acid-hydrolysed casein as a substitute for whole casein

In Exp. 1 the tryptophan-deficient rats failed to recover when tryptophan was added to the diet. Similar anomalies have occasionally been reported. Thus Roche, Roche, Drouineau & Passelaigue (1938) found that rats given a protein-free diet until they lost a quarter of their body nitrogen and then transferred to a diet supplemented with

lysine and containing gliadin and known to support growth in young rats failed to recover. Again, Albanese, Holt, Kajdi and Frankston (1943) found that adult rats given a diet containing acid-hydrolysed casein with 0.225 % L-tryptophan lost weight, and also that three adult rats that had lost weight during a prolonged period of tryptophan deficiency regained weight only slowly and incompletely when tryptophan was added to the diet, although the haemoglobin and plasma-protein levels were fully restored.

Roche & Gueit (1945) showed that the lysine requirement for recovery of adult protein-deficient rats is greater than that of young rats for growth. However, since casein contains nearly three times as much lysine as edestin which was used in Roche & Gueit's experiments, it seems unlikely that a lysine deficiency can have been responsible for the failure of recovery in Exp. 1, nor can a relative tryptophan deficiency have been a major factor in the present work, since doubling the amount in the diet caused no appreciable increase in weight.

According to Morgulis (1923), Avrorov believed that only the terminal stages of inanition are accompanied by pathological changes, and it seems possible that such changes had occurred in the animals of Exp. 1 that had lost an average of 49.1 % of their body-weight, but were absent from those of Exp. 3 that had lost an average of only 35.9 % of their body-weight, and appeared to be in much better condition. Histological examination of the gastro-intestinal tract of the two tryptophan-deficient rats that were, at the time of killing, in a state comparable with that of the deficient rats of Exp. 1 before tryptophan was added to the diet, showed sloughing and necrosis of the villi in the ileum (unpublished data) suggesting that absorption might have been impaired. Sun (1926), however, found that repair of the intestinal villi in starving mice occurred very rapidly when an adequate diet was supplied and that the epithelium had regenerated 10 hr. after refeeding. Though the possibility remains that the amount of food absorbed by the rats of Exp. 1 was inadequate to do more than maintain the animals, it seems unlikely that repair of the intestinal epithelium took 33 days, and that it occurred suddenly just at the time when whole casein replaced the supplemented hydrolysate. Thus, though defective absorption may have been one of the factors involved in the failure of the rats to recover, it seems unlikely that it was the only one.

It seemed possible that an absolute or relative deficiency of some factor such as streptogenin (Sprince & Woolley, 1945) or the 'animal protein factor' (Cary, Hartman Dryden & Likely, 1946) might have been involved, since destruction of these factors would have occurred during the acid hydrolysis of the casein. It is unlikely, however, that this was so since a similar hydrolysate was used in Exps. 3 and 4, in which rapid recovery from tryptophan deficiency and maintenance of adult rats at a satisfactory weight level occurred respectively.

The chief difference between the conditions existing in Exp. 1 and in Exps. 3 and 4 was that in the latter experiments various pure vitamins of the B complex were supplied in addition to the yeast extract, and it seemed possible that the anomalous results might be explicable in terms of the tryptophan-nicotinic acid relationship. The yeast extract given in all experiments supplied about 100 μ g. nicotinic acid/rat/day,

whereas in Exps. 2-4 an extra 1 mg. nicotinic acid/rat/day was given. Under normal conditions the rat does not require dietary nicotinic acid, presumably because it can synthesize sufficient for its own requirements, and recent work suggests that the source may be tryptophan (Rosen, Huff & Perlzweig, 1946). According to Krehl, Henderson, de la Huerga & Elvehjem (1946) nicotinic acid must be supplied at a level of 1.0-1.5 mg./100 g. diet to growing animals receiving an unbalanced mixture of amino-acids, and in Exp. 1 of the present series it may have been that the nicotinic acid supplied by the yeast extract was insufficient for the needs of the animals receiving the deficient diet but, since the animals were losing weight, it is difficult to assess the effect of the amino-acid imbalance on the requirement. However, the greater ability of the rats of Exp. 4 to withstand the effects of tryptophan deficiency as judged by their survival time, more than 100 days as compared with about 63 days in Exp. 1, supports the suggestion of an increased nicotinic-acid requirement even in the absence of growth. If the animals of Exp. 1 were deficient both in nicotinic acid and in tryptophan this might explain the delay in recovery, time being required for the restoration of nicotinic-acid synthesis. However, if such were the case it is remarkable that the re-establishment of the synthetic process coincided with the replacement of diet A.H.C.T. by diet U.C., particularly in view of the fact that tryptophan as such is said to be more effective in promoting nicotinic-acid synthesis than when given in the form of protein (Singal, Briggs, Sydenstricker & Littlejohn, 1946; Bell, Scheer & Deuel, 1948). It is, however, possible that some nicotinic acid may have been supplied by the casein, since the importance of using a vitamin-free preparation was not realized at the time. However, the loss of weight of the rats fed the tryptophan-supplemented diet (A.H.C.T.) in Exp. 1, and the fact that increasing the tryptophan content of the diet from 0.2 to 0.4 % prevented further weight loss still remain unexplained.

The tryptophan requirement of the adult rat

The tryptophan requirement of the young growing rat is reported to be between 0.1 and 0.2 % of the diet, varying slightly according to the other constituents (Berg & Potgieter, 1931-2; Krehl, Sarma & Elvehjem, 1946), whereas between 0.025 and 0.05 % suffices for its maintenance (Jackson, 1929; Berg, Rose & Marvel, 1929-30; Berg & Potgieter, 1931-2). These levels are equivalent to intakes of about 10 and 2.5 mg./100 g. rat/day for growth and maintenance respectively.

Although the number of animals used was small, the results of Exp. 4 suggest that maintenance of adult female rats after recovery from tryptophan deficiency may be achieved over a fairly wide range of tryptophan intake, but the weight at which the animals are maintained may vary with the level of tryptophan in the diet. Thus there was a direct correlation between the percentage of the initial weight recovered and the logarithm of the percentage of tryptophan in the diet up to the 0.1 % level as shown in Fig. 7. Above this the rate of recovery decreased, suggesting that a level of between 0.1 and 0.2 % is sufficient for maintenance at a maximal weight level on the supplemented A.H.C. diet and that the percentage dietary requirement for tryptophan of the adult female rat after an initial period of tryptophan deficiency is similar to that of the young growing rat. During the later weeks of the experiment when a steady weight

level had been reached the actual amounts of tryptophan consumed by the rats at the 0.2, 0.1 and 0.05 % levels averaged 12.2, 5.8 and 3.3 mg./rat/day respectively. The group given tryptophan at the 0.01 % level lost weight continuously.

This finding does not agree with that of Albanese, Holt, Irby, Snyderman & Lein (1947) that the daily tryptophan requirement of the infant is about 30 mg./kg. body weight, as compared with a previously determined adult human requirement of 6 mg./kg. (Holt, Albanese, Frankston & Irby, 1944). It is also in marked contrast with the findings of Neuberger & Webster (1945) for lysine that the ratio of the growth requirement of the young rat to the maintenance requirement of the adult was of the order of 6:1, or even higher if the comparison was made on the basis of body surface

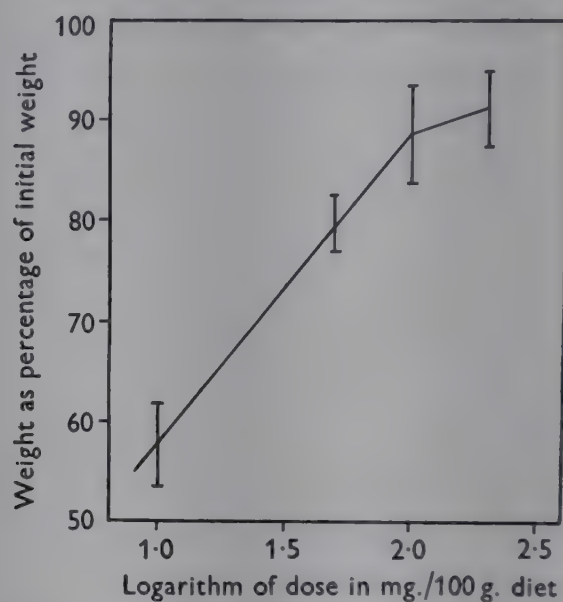


Fig. 7. Exp. 4. Relationship of the weight, as a percentage of the initial weight, of the rats to the logarithm of the dose of tryptophan in mg./100 g. diet.

instead of body-weight. This may indicate that, whereas lysine is required by the adult only for the replacement of endogenous losses, tryptophan performs some additional function, or alternatively that the rate of destruction of tryptophan is very rapid in both the young and adult rat. This latter possibility is suggested by the fact that the amount of ingested tryptophan retained as such in the tissue proteins and body fluids (Fürth & Lieben, 1922) and excreted (Schweigert, Sauberlich, Elvehjem & Baumann, 1946) is low and that it disappears rapidly from the blood after ingestion (Buck & Berg, 1945).

However, since three out of four rats lost weight when tryptophan was given at the low level of 0.01 % of the diet as rapidly as when no tryptophan was supplied, it seems possible that the available tryptophan was used in the performance of special functions, leaving no residue to supply the tissues generally. If this were the case, some tissue or function should have been better maintained in those rats than in the completely deficient ones. The degree of anaemia in the rats given 0.01 % tryptophan was less severe than in those given no tryptophan, and it is hoped that histological examination of tissues may show further differences between the two groups.

SUMMARY

1. The gross symptoms of tryptophan deficiency in the adult female rat were found to be similar to those of protein deficiency. In addition, there was necrosis of the tail and accumulation of porphyrin-like material on the nose and paws. The animals lost about 50 % of their body-weight and survived for periods up to 112 days. Oestrus was inhibited during the deficiency, but even when this was severe, permanent sterility was not induced. Marked anaemia was observed in the deficient rats.

2. The ability of acid-hydrolysed casein supplemented with tryptophan to maintain adult female rats, to promote growth in young rats and to afford recovery from tryptophan deficiency was studied. In one experiment with tryptophan included at a level of 0.2 %, the diet failed to maintain groups of adult rats, and in a further group it failed to support recovery from severe tryptophan deficiency. In a later experiment a similar diet allowed recovery from a moderate degree of deficiency, and thereafter animals were maintained at varying weight levels when tryptophan was fed at levels of 0.05, 0.1 and 0.2 % of the diet. The possible reasons for this discrepancy are discussed.

3. The optimal level of tryptophan in the diet of the adult female rat was found to be between 0.1 and 0.2 %, which is similar to that previously reported for the young growing rat.

We wish to record our thanks to Glaxo Laboratories Ltd. for their gift of acid-hydrolysed casein, to Mr S. Thurlow for preparing much of the tryptophan used in these experiments and to Dr M. Ginsburg who made the statistical analyses.

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Induced Cobalt Deficiency in Lambs

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The object of the experiments described below was to induce cobalt deficiency in lambs by giving a diet composed of foodstuffs sufficiently remote from pasture to avoid the criticism that some unknown pasture factor other than cobalt might be involved in the causation of the marasmus, now called 'cobalt deficiency', and to prove conclusively that a lack in the diet of cobalt *per se* is responsible for 'pining' in lambs.

EXPERIMENTAL

First experiment, 1946

Basic ration. The basic daily ration used was 1100 g. flaked maize, 250 g. hay and 60 g. of a mineral mixture composed of ground limestone, steamed bone-flour and crude rock salt. The introduction of hay was necessary to ensure that the rumen processes would be normal, as it was not considered satisfactory to use wood pulp or a similar source of cellulose, since it might cause upsets in rumination, regarding which little is known. The hay was kept to a minimum (250 g./day) and was obtained from a field of which the soil was deficient in cobalt.

The diet was adequate in starch and protein equivalent for a pregnant or lactating ewe. According to Woodman (1948), a 120 lb. ewe requires 10 lb. starch equivalent (S.E.)/week for maintenance and 4 lb. starch equivalent (S.E.)/week/gal. milk; and 0.46 lb. protein equivalent (P.E.)/week for maintenance and 1 lb. protein equivalent (P.E.)/week/gal. milk. The starch equivalents of the flaked maize and hay were 84 and 30 respectively, and the protein equivalents were about 10 and 3. Thus the diet outlined above supplied the energy requirements for maintenance and for at least 1 gal. weekly of milk, the average weight of the ewes being 115 lb.

The cobalt content of the ration was assayed spectrographically with the following result:

Flaked maize	0.025 p.p.m.
Hay	0.10 p.p.m.
Mineral mixture	0.27 p.p.m.

Thus the daily intake of cobalt/ewe was about 0.0687 mg. Little is known of the daily requirements of sheep for cobalt. Australian research workers (Filmer & Underwood, 1937) have reported cure and prevention of cobalt deficiency by dosing with as small an amount of cobalt as 0.1 mg./day. The cobalt content of the Scottish pastures on which lambs pine ranges from 0.10 to 0.03 p.p.m. (Stewart, Mitchell & Stewart, 1941), and if lambs eat as much as 2.5 lb. dry matter daily they would be ingesting 0.1135–0.034 mg. cobalt. It was considered possible that the experimental diet containing 0.0687 mg./day might be low enough to produce pine in lambs.

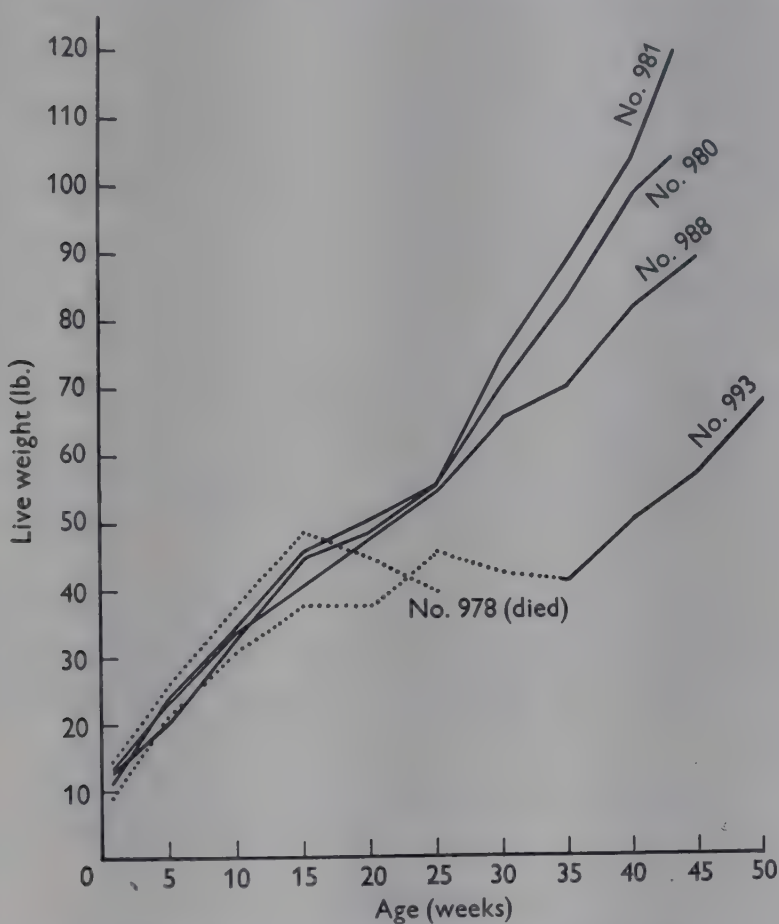


Fig. 1. Live-weight curves of lambs in the 1946 experiment. —, lambs receiving cobalt-deficient diet and a cobalt supplement;, lambs receiving cobalt-deficient diet only.

Sheep. On 1 January 1946 nine in-lamb ewes were divided into three equal groups and placed in boxes with cement floors covered with peat-moss litter. They were fed the above ration, but each member of one group received weekly 10 mg. of cobalt, as cobalt chloride.

The ewes ate the ration readily, but several deaths occurred during the last week of pregnancy due to 'lambing sickness', probably brought about by the nature of the diet, highly digestible flaked maize, and by the lack of exercise due to restraint of the stalls.

Lambs. There were eventually on experiment five lambs, three, nos. 980, 981 and 988, from control ewes receiving the diet and 10 mg. cobalt/week and two, nos. 978 and 993, from the ewes receiving the diet only. Nos. 980, 981 and 988 received 10 mg. cobalt/week from birth and, when weaned at 10 weeks of age, were given 800 g.

flaked maize, 80 g. hay and 25 g. mineral mixture daily, which supplied 0.0347 mg. cobalt. The ration was steadily increased till at 16 weeks of age the lambs were being offered the full amount given to their mothers. This appeared to be the limit of appetite and occasionally food was left uneaten, so that the cobalt ingested from the diet was never above 0.0687 mg./day.

Further experiments, 1947-8

Basic ration. The basic ration was the same as in the first experiment.

Despite the foodstuffs being from new sources the cobalt content was still about 0.0687 mg./day, compensation for any increase in the cobalt content of the feeding-stuffs being made by altering slightly the amount of mineral mixture fed. Several of the ewes died of 'lambing sickness' in both years, but in similar experiments in 1949 and 1950, when great care was taken to exercise the ewes once a week by allowing them to run in concrete yards, no deaths occurred, the ewes all remaining healthy and lambing successfully. It is concluded that in the previous years the deaths in ewes on this diet were caused by the enforced lack of exercise rather than by the composition of the ration. Several lambs died in both 1947 and 1948 in the neonatal stage from a pyaemia caused by a staphylococcal infection.

Lambs. Lambs nos. 334, 338, 339, 340, 768, 789 and 806 were from ewes receiving cobalt supplement and lambs nos. 335, 336, 345, 785, 787, 788, 800 and 816 were from ewes receiving the diet only.

Lamb no. 816 within a few days after birth showed signs of a staphylococcal infection, and shortly afterwards its mother died. From this stage it was raised on cow's milk and transferred to the control group receiving 10 mg. cobalt weekly after the 3rd week of life.

The lambs were managed exactly as in 1946 but fed individually.

RESULTS

First experiment, 1946. Lambs nos. 980, 981 and 993, receiving 10 mg. cobalt/week, made steady gains in weight, as seen in Fig. 1. Their live-weight curves correspond to those of lambs on good pasture. Lambs nos. 978 and 993 were offered the same diet as the others, and it will be seen from Fig. 1 that they showed similar weight gains until about the 14th week of age, no. 978 being the heaviest of all the five lambs at that age. From the 15th week no. 978 began to lose weight steadily and died at the 25th week, showing all the symptoms of cobalt deficiency and weighing only 38 lb. The weight of no. 993 from the age of 14 weeks fluctuated round 40 lb. and at 31 weeks was only 46 lb., as compared with 66, 72 and 75 lb. for nos. 980, 981 and 988 respectively. From this age no. 993 was dosed with 10 mg. cobalt weekly. Its weight, after falling for the 1st week, rose steadily and at 48 weeks of age was 68 lb., an increase of 27 lb. in 17 weeks, the live-weight curve being parallel to that of the control lambs.

Lambs nos. 978 and 993 had smaller appetites than the controls. They were continually leaving food, and various methods were tried to persuade them to eat more. Neither ever ate the full ration of flaked maize, hay and mineral mixture, so that their

cobalt intake was always less than 0.0687 mg./day. When lamb no. 993 was given cobalt at 31 weeks old it began to eat its ration more quickly, but even at 48 weeks it still left a quantity in the feeding trough.

Further experiments, 1947-8. The live-weight curves of the lambs, with the exception of nos. 787 and 788, are shown in Fig. 2. Lamb no. 816 made little progress for the first 10 weeks but from that age made normal live-weight gains, reaching 75 lb. at the 35th week. Lamb. no. 340, which had never made normal live-weight gains, died at 18 weeks of age. A post-mortem examination failed to reveal the cause of death.

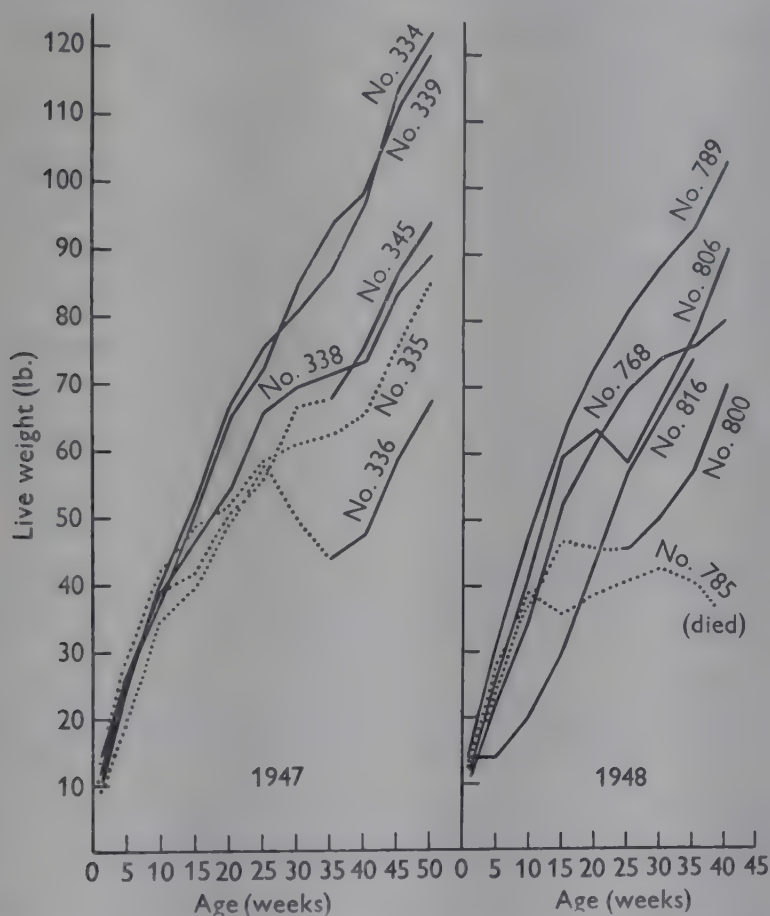


Fig. 2. Live-weight curves of lambs in the 1947 and 1948 experiments. —, lambs receiving cobalt-deficient diet and a cobalt supplement;, lambs receiving cobalt-deficient diet only.

All the control lambs receiving 10 mg. cobalt/week from birth made normal live-weight gains, with the exception of no. 338 which weighed only 74 lb. at the 40th week. Lamb no. 806 fluctuated in weight after the 15th week, but by the 25th week resumed gaining weight in a normal manner. The lambs on deficient diet only showed the effects of this diet at the 15th week. Lamb no. 785 continued on the deficient diet, but died at the 38th week with all the clinical symptoms of cobalt deficiency and weighing only 38 lb. The other lambs in this group received cobalt at different stages once they had obviously shown that their weight curves were on the decline, and immediately made spectacular increases in weight. Anomalous behaviour was shown by lamb no. 335. This animal at 25 weeks of age weighed only 60 lb. and appeared likely to become a typical case of cobalt deficiency if left on the deficient diet. No cobalt was given but, despite this, it began to gain weight and at 50 weeks of age

weighed 85 lb. There is no accountable reason for this behaviour of no. 335 except that the diet of 0.0687 mg. cobalt must be near the margin of adequacy and that for exceptional animals this amount is sufficient to allow them to keep their appetite and so maintain, or even gain, weight.

Lambs nos. 787 and 788 were twin lambs of a ewe on the deficient diet only. Their weight curves are shown in Fig. 3.

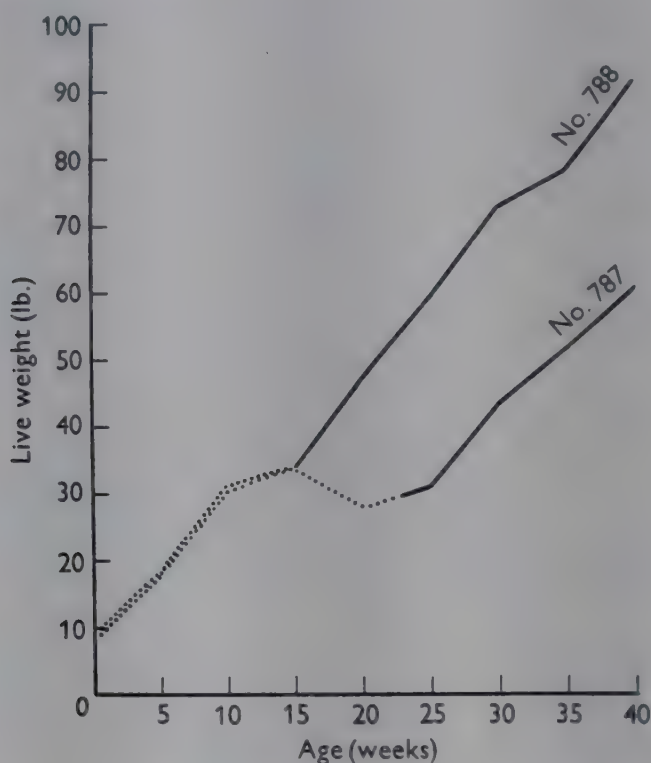


Fig. 3. Live-weight curves of twin lambs nos. 787 and 788. —, receiving cobalt-deficient diet and a cobalt supplement;, receiving cobalt-deficient diet only.

They made identical weight gains till 15 weeks of age, when each weighed 34 lb. At this age no. 788 received 10 mg. cobalt weekly and increased its weight by 21 lb. in 8 weeks, whereas no. 787, receiving no cobalt, lost 6 lb. in the same period. At the 23rd week no. 787 was also given cobalt at the rate of 10 mg. weekly and in the next 17 weeks added 32 lb. to its weight, whilst no. 788 added 35 lb.

DISCUSSION

Three postulates must be fulfilled to justify the statement that a deficiency of a particular constituent of the diet is the cause of a definite disease syndrome in a certain species of animal.

(1) It should be possible to correlate every case of the disease with a real deficiency of the particular constituent in the diet under consideration.

(2) Addition of the particular constituent to the deficient diet should cure or prevent the disease.

(3) The disease condition should be capable of induction in healthy animals by the feeding of an artificial diet, adequate in every essential constituent except the particular constituent, which should be of a content comparable to that in the diet of naturally occurring cases of the disease.

Of these postulates, no. 3 has been often ignored of recent years, with the result that we are still in doubt regarding the causal role of many dietary constituents in well-established disease syndromes, and this is especially so with regard to the trace elements. It is not sufficient to use the criterion of (1) low intake, (2) depletion of the animal, as shown by tissue analysis, and (3) dramatic response after addition of a constituent to the diet or by injection of the animal, since the interrelationships of trace elements one with another have confused the true significance of the part played by several trace elements in disease syndromes, e.g. copper and molybdenum in 'teart' disease, copper and cobalt in 'coast' disease and the anomalous position of copper in the prevention of swayback in lambs in Great Britain, which usually occurs on pastures of normal copper content.

Several Australian workers (Marston, 1949) have produced the syndrome of cobalt deficiency by cutting herbage from a suspected cobalt-deficient area and feeding it to sheep kept indoors, and Bowstead and his colleagues in Canada (Bowstead & Sackville, 1939; Bowstead, Sackville & Sinclair, 1941-2), by maintaining sheep on a basal diet of non-leguminous hay and ground oats, reproduced a condition of unthriftiness which, after many curative attempts with a large number of substances, all without real effect, responded to cobalt therapy, but again the foodstuffs fed were all harvested off the suspected soil. Therefore neither of the above types of investigation fulfils postulate no. 3, since the ration used does not rule out the presence of some still unknown factor causing the physiological upset curable by cobalt supplements, similar to the action of copper in preventing molybdenum poisoning in 'teart' disease.

The experiments described in this paper therefore were attempts to fulfil the third postulate as far as cobalt deficiency was concerned as a causal factor of 'pine' in lambs and, although hay from a cobalt-deficient area was used, it was kept to a minimum, being only one-sixth of the total dry matter fed.

The majority of the experimental animals receiving the basal diet and cobalt made the normal live-weight gains to be expected from the starch equivalent and protein equivalent of the ration, reaching approximately 100 lb. live weight in about 40 weeks. Those receiving no cobalt supplement made normal progress till about the 10th-14th week when they had reached a weight of 40 lb., then their weight remained steady or decreased and, if continued on the deficient basal diet, the lambs showed eventually the signs of marasmus identical with that attributed to cobalt deficiency. The live-weight curves of both the control and the experimental animals were very similar to those obtained in experiments carried out in the field (Stewart, 1946). There was a dramatic response with every deficient lamb given cobalt, the two outstanding examples being the twins, nos. 787 and 788.

It would appear that the diet designed for these experiments which allowed the lambs a maximum cobalt intake of 0.0687 mg./day will produce the symptoms of cobalt deficiency, and that an immediate recovery response is obtained by allowing the marasmic lambs to ingest cobalt *per se*.

This type of diet might be of great value in the production of cobalt deficiency in experimental lambs and should facilitate work being carried out on the physiological action of cobalt and other research on cobalt deficiency.

SUMMARY

1. In-lamb ewes were fed a diet of 1100 g. flaked maize, 250 g. hay and 60 g. mineral mixture daily for the last 3 months of pregnancy. This diet allowed of a daily ingestion of 0.0687 mg. cobalt. Their lambs when weaned at 10 weeks of age were given 800 g. flaked maize, 80 g. hay, 25 g. mineral mixture daily (0.0347 mg. cobalt) increasing steadily to the quantities in the ewe's diet at 16 weeks of age. These lambs all eventually showed the clinical signs of cobalt deficiency but made immediate dramatic response if fed 10 mg. cobalt/week.
2. Lambs given the same basal diet with a supplement of 10 mg. cobalt from birth all gained weight normally.
3. The necessity for this type of experimentation in trace-element research is stressed.
4. The usefulness of a diet such as that described for the production of cobalt deficiency to facilitate work on the physiological action of cobalt and other research on cobalt deficiency is demonstrated.

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The Nutritional Status of Cook Islanders

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Several observers have noted profound changes in the diet of the Rarotongan since the advent of the European about 100 years ago (Davis, 1947; Abraham, 1947; Peren, 1947; Wingfield, 1949).

Although native local foods still remain the staple diet, increasing reliance is being placed on imported European-type food, chiefly meat, flour, sugar and biscuits. With a view towards assessing the effects of these changes and the adequacy of the diet as a whole, nutritional status was investigated in the course of a survey of the health of Cook Islanders carried out in January and February 1950.

METHODS

The subjects of this survey were 365 people of all ages, chosen at random in sixty-six family groups from the village of Arorangi, on the island of Rarotonga, in the Cook Islands. As seen from Table 1, they comprised a representative sample (about one-third) of the population of the village of Arorangi, and in turn a representative sample of the whole island's population of approximately 6000.

Table 1. Age and sex distribution of subjects of survey and of population of Arorangi village and of Island of Rarotonga

Age (years)	Subjects of survey 1950						Arorangi 1945 census						Rarotonga 1945 census (%)
	Male		Female		Total		Male		Female		Total		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Under 5	26	14.2	29	15.9	55	15.1	88	15.1	96	17.9	184	16.5	15.9
5-9	22	12.0	34	18.7	56	15.4	118	20.3	106	19.6	224	19.9	15.7
10-14	41	22.4	34	18.7	75	20.6	109	18.7	84	15.7	193	17.2	13.6
15-19	24	13.1	12	6.6	36	9.8	58	10.1	42	7.8	100	8.9	9.8
20-29	15	8.2	20	11.0	35	9.6	69	11.9	73	13.6	142	12.3	15.0
30-39	27	14.8	21	11.6	48	13.2	55	9.5	55	10.4	110	10.0	10.9
40-49	12	6.5	16	8.8	28	7.6	32	5.5	27	5.1	59	5.3	8.3
50-59	10	5.5	11	6.0	21	5.7	28	4.8	24	4.5	52	4.6	6.0
60 and over	6	3.3	5	2.7	11	3.0	24	4.1	29	5.4	51	4.7	4.8
Total	183	100.0	182	100.0	365	100.0	581	100.0	536	100.0	1117	100.0	—

By occupation, fifty of the adults were planters entirely dependent upon the products of their plantation, thirty-one worked regularly for wages in addition to planting, and twenty-one were entirely dependent upon wages which averaged about 6s. a day. Of

the group, 67 % were full Polynesian, 15 % from a quarter to half European, and 11.5 % half or more European.

There is no satisfactory single index of nutritional status. Methods that measure 'nutritional status', or any or some of its various components, may be either subjective or objective or both.

Clinical methods

The methods used included a general clinical examination and an assessment ('subjective nutritional assessment') arrived at by roughly averaging marks (ascending from 1 to 5 with improving condition) for each of skeletal development, muscular development and tone, quality and quantity of subcutaneous tissue, skin condition and general appearance. Marks were deducted for each defect from a possible 5 in each category, according to a definite scale.

Dental state

A full dental examination was made and the condition of the teeth recorded according to a modification of the Medical Research Council system (Mellanby, 1934).

Anthropometric measurements

Objective measurements of various components of nutritional status were made as follows:

Height was measured without shoes to the nearest $\frac{1}{4}$ in.

Weight was taken on a beam balance without shoes but with clothes (always very light), in lb.

The weight: height ratio ($W:H$) was calculated as weight in lb. divided by the height in inches.

The Tuxford index was calculated after Tuxford (1942) from $\frac{W}{H} \times \frac{336-m}{270}$ for boys, and $\frac{W}{H} \times \frac{308-m}{235}$ for girls, where m is the age in months.

Laboratory tests

Haemoglobin was measured both colorimetrically by the Hellige apparatus modification of Sahli's method, and by calculation from blood specific gravity (Phillips, Van Slyke, Dole, Emerson, Hamilton & Archibald, 1945; Van Slyke, Phillips, Dole, Hamilton, Archibald & Plazin, 1950).

Erythrocytes were counted by standard methods.

Plasma protein was measured in oxalated plasma by the copper-sulphate specific gravity method of Phillips *et al.* (1945) and of Van Slyke, Hiller, Phillips, Hamilton, Dole, Archibald & Eder (1950). The formula,

$$\text{protein (g./100 ml.)} = 364 (G_p - 1.006),$$

where G_p is the observed specific gravity, was used, following Hoch & Marrack (1945).

This gives values for protein approximately 0.15 g./100 ml. higher over the range, than the formula, 373 ($G_p - 1.007$), recommended by Van Slyke, Phillips *et al.* (1950).
Sedimentation rate was measured by Wintrobe's method (Wintrobe, 1935).

Dietary assessment

A qualitative assessment of the diet of each family was made by interrogation and observation.

Vital statistics

The vital statistics for the Cook Islands as a whole and for Rarotonga were considered.

RESULTS

Clinical methods

General physical examination. On the whole the people appeared reasonably clean, cheerful and well nourished. Almost without exception their co-operation was good. There was one case of malnutrition, in an infant of 8 months, following a bout of unspecified diarrhoea several weeks previously. The Mantoux test was negative, but the home conditions and parental education were among the poorest seen. The only deformities seen were due to trauma, or had some explanation other than malnutrition. No clinical signs of malnutrition were noted. Only one case of thyroid enlargement of a minor nature was seen.

Skin conditions. Scars, of both infective and traumatic origin, were encountered almost universally; scabies, head-lice and ringworm were also common. No skins were seen that reflected nutritional deficiency.

Table 2. Subjective nutritional assessment of subjects of survey based on assignment of marks from 1 for poor to 5 for excellent for each of skeletal development, muscular development and bone, quality and quantity of subcutaneous tissue, skin condition and general appearance

Age (years)	No. examined	Marks									
		1		2		3		4		5	
		No.	%	No.	%	No.	%	No.	%	No.	%
0-2	34	1	3.2	8	23.5	14	41.1	11	32.2	—	—
3-5	32	—	—	3	9.4	13	40.6	16	50.0	—	—
6-12	90	—	—	6	6.6	49	54.4	35	38.8	—	—
13-20	68	—	—	4	6.5	19	27.9	44	64.7	1	1.4
21-45	98	1	1.0	4	4.0	43	43.8	49	50.0	—	—
Over 45	43	1	2.3	6	13.9	23	53.5	13	30.2	—	—
Total	365	3	0.8	31	8.2	161	44.1	168	46.0	1	0.3

Subjective nutritional assessment. The results are shown in Table 2. It will be seen that most people are graded 3-4, that is, average to good. Only one person was so outstanding as to deserve 5, and only three so poor as to deserve 1; two of these suffered disability following accidents. Posture was remarkably good, as was muscular

development. Obesity was the rule, especially in women, as seen from the height-weight graphs (see below).

Dental and oral examination

The results of the dental examination are shown in Tables 3 and 4. It will be seen that of the 361 mouths examined, twenty-four were edentulous, eight on account of infancy, sixteen had had all their teeth extracted and of these nine had no dentures. Of the remaining 337, twelve (4 %) were free from dental caries (ten of these were 1 year old or less, one was aged 3, and one 15). Of the 325 with dental caries, 258 (80 %) were over 6 years of age. The table shows that there was no important difference between the incidence in the various age groups.

The degree of caries and the number of fillings are shown in Tables 3 and 4 by age groups. It is evident that the severity of the caries increases as age advances. It is noteworthy that only one individual over 40 had any fillings; this man had been overseas with the Cook Island contingent to the first World War and the copper-amalgam fillings were the work of the dental unit at Moascar Camp. Most of the dental work had been carried out by the School Dental Service but in few mouths had the necessary dental work been completed. Out of sixteen totally edentulous people seven had full dentures, and two out of nine without upper teeth had full upper plates and six others had partial upper plates. The same type of anomalies was found as was mentioned by Wingfield (1949). This hypoplasia of enamel was found in a number of cases. In one family all members were affected. A number of cases of cracked enamel on the palatal surfaces of the upper incisors was noted, and marked attrition on the occlusal surfaces of the molars with exposure of dentine—such teeth were free of caries. The use of a toothbrush is being introduced among schoolchildren.

Health of gums. The incidence of gingival disease by age groups in 343 individuals in this study contrasted with the number of teeth present in the mouth is shown in Table 5. The steadily increasing amount and degree of gingival disease with advancing age is evident.

Health of pharynx. Table 6 shows that the state of health of the throat and tonsils was excellent.

Anthropometric measurements

Height and weight. The average height and weight for each age group are shown in Tables 7 and 8; in addition, the weights in relation to height, for males and females irrespective of age, are shown in Fig. 1. The heights and weights of Rarotongan children studied in this group are compared with similar figures for children of comparable age from other countries in Tables 9 and 10.

In an attempt to standardize figures to a comparable base, weight: height ($W:H$) ratios and the Tuxford index (Tuxford, 1942) for the groups shown in Tables 9 and 10 have been calculated and are presented in Table 11 and Fig. 2. It will be seen from all these figures that, although the samples are small, Rarotongan children are of approximately the same build, height and weight as New Zealand European children, smaller than New Zealand Maoris, but bigger than the other groups in most respects.

Table 3. Teeth and incidence of caries among subjects of survey

Age (years)	No. of people examined	No. of No. of with erupted teeth	No. of edentulous or with un- erupted teeth	Total no. of teeth	Av. no. of teeth per person	Caries teeth										Av. no. of caries teeth/ person with caries	Cari- ous teeth as per- centage of total
						People with caries		1		2		3		4			
						No.	%	No.	%	No.	%	No.	%	No.	%		
						Dentition: deciduous											
						Dentition: deciduous and permanent											
0-5	57	49	8	944	19.3	45	91.8	205	73.7	56	20.1	15	5.3	2	0.7	278	29.4
6-12	57	57	0	568	10.0	48	84.2	120	48.4	82	33.1	32	12.8	14	5.6	248	43.7
0-5	5	5	0	90	18.0	5	100	29	48.4	19	31.8	11	18.3	1	1.6	60	66.6
6-12	89	89	0	2088	23.5	87	98	432	70.4	126	20.9	38	6.2	19	3.1	614	29.4
0-5	5	5	0	22	4.5	3	60	8	—	1	—	—	—	—	—	9	41.0
6-12	84	84	0	1516	18.1	77	91.5	303	84.1	50	13.7	2	0.6	5	1.4	360	23.7
13-20	66	66	0	1823	27.6	63	92.8	404	71.4	144	25.2	13	2.3	7	1.2	572	31.4
21-40	90	83	7	2147	25.9	81	97.5	597	61.1	217	22.2	30	3.1	133	13.6	977	45.5
41-50	20	18	2	433	24.1	18	100	94	39.7	39	16.4	50	21.0	54	23.0	237	54.7
Over 50	26	19	7	334	17.6	19	100	70	40.5	37	20.2	20	11.6	46	25.6	173	51.8

Table 4. Fillings and extractions of teeth among subjects of the survey

Age (years)	No. of people (with teeth) examined	People with fillings		No. of teeth examined	Teeth filled		Av. no. of fillings/ person with fillings	No. of teeth extracted or missing
		No.	%		No.	%		
0-5	49	0	0	944	0	0	0	7
6-12	57	25	43.8	568	55	9.7	2.2	98
Dentition: deciduous								
0-5	5	0	0	90	0	0	0	7
6-12	89	59	66.2	2088	169	8.1	2.9	113
Dentition: deciduous and permanent								
Dentition: permanent								
0-5	5	0	0	22	0	0	0	0
6-12	84	50	59.5	1516	122	8.0	2.4	16
13-20	66	42	63.6	1823	148	8.1	3.5	53
21-40	83	25	30.1	2147	88	4.1	3.5	670
41-50	18	0	0	433	0	0	0	197
Over 50	19	1	5.3	334	1	0.03	1	476

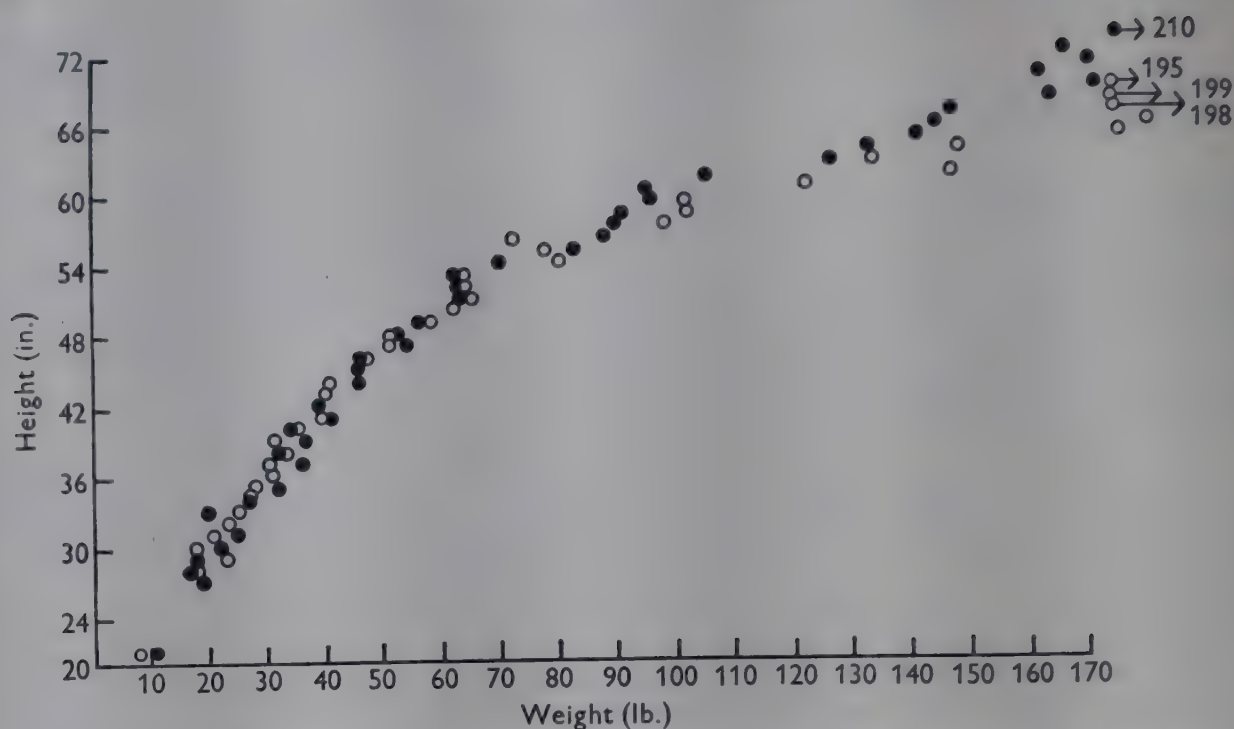


Fig. 1. Average weight in lb. of sample of population of Arorangi, classified according to height in inches. ●, males; ○, females.

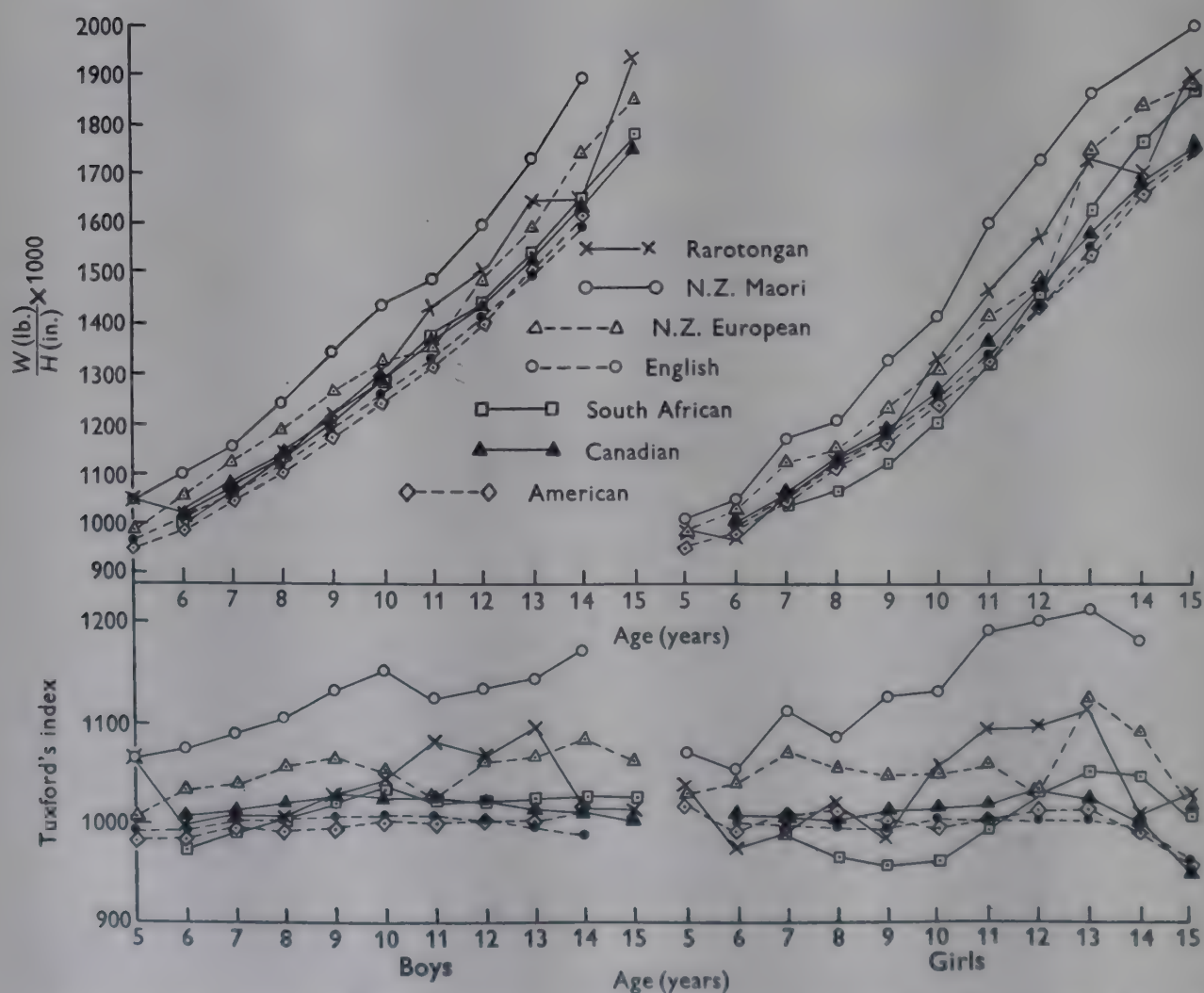


Fig. 2. Weight:height ratio and Tuxford's index for children measured on Rarotonga, classified according to age and compared with published data for children of other races.

Table 5. Condition of gums of subjects of survey

Age (years)	No. of people (with teeth) examined	People with gingivitis in grade*											
		0		1		2		3		4		5	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0-5	66	62	93.9	3	4.5	1	1.5	—	—	—	—	—	—
6-12	90	80	88.9	9	10.0	1	1.1	—	—	—	—	—	—
13-20	66	47	71.2	11	16.7	5	7.7	3	4.5	—	—	—	—
21-40	83	12	14.5	23	27.8	22	26.4	21	25.3	6	7.3	—	—
41-50	18	0	0	4	22.2	6	33.3	5	27.8	2	11.1	1	5.5
Over 50	20	1	5.0	0	0	4	20.0	7	35.0	5	25.0	3	15.0
Total	343	—	—	—	—	—	—	—	—	—	—	—	—

* 0: gums healthy; 1: commencing gingivitis; 2 and 3: progressively increasing gingivitis and early pyorrhoea; 4 and 5: severe and gross pyorrhoea.

Table 6. Condition of throat and tonsils of subjects of survey graded in age groups

Age (years)	Total no. examined	Grading*					
		0		1		2	
		No.	%	No.	%	No.	%
0-5	66	58	88.0	6	9.1	2	3.0
6-12	90	74	82.3	12	13.3	4	4.4
13-20	66	54	81.7	10	15.3	2	3.0
21-40	90	79	87.8	11	12.2	—	—
41-50	20	18	90.0	2	10.0	—	—
Over 50	29	25	86.2	4	13.8	—	—

* 0: healthy throat and tonsils; 1: enlargement of tonsils; 2: enlarged tonsils and infected throat. No cases of tonsillar enlargement or unhealthy throats beyond this degree were recorded.

Table 7. Ages and heights of subjects of the survey

Age (years)	Males					Females				
	No. examined	Height				No. examined	Height			
		Average ft. in.	Range ft. in.				Average ft. in.	Range ft. in.		
2	5	3 0	2 7	to 3 1	5	2 10½	2 8	to 3 3		
3	4	3 0	2 9	to 3 3	7	3 1½	2 9	to 3 5		
4	5	3 3	3 1	to 3 4	4	3 4	3 4	to 3 5		
5	6	3 6	3 1	to 3 9	5	3 4½	3 4	to 3 5		
6	4	3 7½	3 5	to 3 11	13	3 9	3 7	to 4 1		
7	3	4 0	3 10	to 4 1	6	3 10½	3 9	to 4 1		
8	6	4 0½	3 10	to 4 4	3	4 0½	4 0	to 4 1		
9	3	4 4½	4 3	to 4 4	6	4 2	4 0	to 4 3		
10	8	4 4	4 1	to 4 7	7	4 5	4 3	to 4 10		
11	6	4 7½	4 4	to 4 10	10	4 7½	4 1	to 5 0		
12	10	4 8½	4 4	to 5 2	4	4 10½	4 5	to 5 1		
13	6	5 0	5 1	to 5 3	8	4 11½	4 4	to 5 3		
14	10	5 4	4 10	to 5 4	5	5 0½	4 10	to 5 4		
15	9	5 4	5 0	to 5 6	2	5 1½	4 11	to 5 4		
16	4	5 7	5 5	to 5 9	2	5 3½	5 3	to 5 4		
Over 16	82	5 6½	5 1	to 6 0	81	5 3	4 9	to 5 8		
Total	171	—	—	—	168	—	—	—		

Table 8. *Ages and weights of subjects of the survey*

Age (years)	Males			Females*		
	No. examined	Weight		No. examined	Weight	
		Average (lb.)	Range (lb.)		Average (lb.)	Range (lb.)
Under ½	4	15·3	11·2- 18·2	4	13·0	8·4- 18·2
½-1	0	—	—	3	18·2	16·8- 19·6
1	5	20·2	16·8- 22·4	5	21·3	18·2- 26·6
2	5	28·3	32·2- 33·6	5	26·3	21·0- 30·8
3	4	23·5	23·0- 37·8	8	32·1	25·2- 37·8
4	5	34·3	32·2- 35·0	4	35·3	32·2- 39·2
5	6	43·8	39·2- 49·0	4	39·9	36·4- 44·8
6	4	44·5	37·8- 67·2	13	43·7	33·6- 54·6
7	3	51·8	49·0- 54·6	6	48·4	43·4- 58·8
8	6	54·6	43·4- 67·2	3	55·0	51·8- 57·4
9	3	64·0	63·0- 64·4	6	58·1	50·4- 67·2
10	8	67·9	58·8- 85·4	7	70·0	57·4- 85·4
11	6	79·2	60·2-106·4	11	81·1	54·6-114·8
12	10	84·6	57·4-116·2	4	92·0	60·2-113·4
13	6	98·5	91·0-106·4	8	102·5	79·8-130·2
14	10	104·6	81·2-131·6	5	102·5	85·4-130·2
15	9	123·3	100·8-148·4	2	116·2	102·2-130·2
16	4	140·0	121·8-165·2	2	131·6	126·0-137·2
17-20	12	143·5	123·2-172·2	9	137·6	100·8-214·3
21-30	17	159·3	128·8-191·8	18	154·1	100·8-214·2
31-40	29	172·9	114·8-226·8	24	117·2	117·6-225†
41-50	9	172·5	148·4-240·8	10	170·2	105·0-253·4
51-60	15	165·1	116·2-252·0	14	162·5	113·4-235·2
Total	175	—	—	180	—	—
20-50 years (with children)				48	167·4	113·4-225†
20-50 years (without children)				9	170·8	102·2-196

* Excluding pregnant women and those with gross elephantiasis.
† Apart from one person more than 280 lb. weight the highest weight was 225 lb.

Laboratory tests

Haemoglobin and erythrocyte count. The results of the haemoglobin determinations are shown in Table 12. The mean haemoglobin level with its standard error of all the subjects tested, both males and females, was 14·18 ± 0·09 g./100 ml. blood as measured colorimetrically. Calculated from the specific gravity of the blood, a mean level of 14·41 ± 0·07 g./100 ml. blood was obtained. Although the results by these two methods did not always coincide, there was a significant correlation of $r = 0·475 \pm 0·045$ between these two means. There was a significant difference between males and females in the 16-45 age group, although the absolute values were normal. Erythrocyte counts on 149 subjects gave a mean level of 4·55 ± 0·07 million/cu.mm. The lowest reading found was 3 million cu.mm.

Plasma protein. The results of the determinations of plasma protein are shown in Table 13. The levels generally were high, and increased with age. This increase commenced early, as three blood samples from the youngest investigated—in the 1-3 years age group—were over 6·5 g./100 ml.; although this was within generally accepted limits, there was a distinct upward trend with age.

Table 9. Comparison of average heights (in in.) of Rarotongan children measured during the survey with those of New Zealand (Maori and European), English, South African, Canadian and American children of similar age

	Age (years)										
	5	6	7	8	9	10	11	12	13	14	15
Boys											
Rarotongan	42.0	43.5	48.0	48.25	52.5	52.0	55.25	56.25	60.0	64.0	64.0
New Zealand: Maori*	43.75	45.60	48.15	50.25	52.21	54.20	55.80	57.79	60.14	62.98	—
European*	44.28	46.44	48.38	50.53	53.07	54.59	55.43	58.15	60.06	62.53	65.27
English†	—	44.1	46.3	48.5	50.6	52.5	54.4	56.2	57.8	59.4	—
South African‡	—	46.0	48.0	50.0	52.0	54.0	56.0	58.0	60.0	62.3	64.6
Canadian§	—	45.1	47.4	49.6	51.7	53.6	55.4	57.4	59.4	61.6	63.9
American	—	45.2	47.5	49.6	51.7	53.6	55.4	57.3	59.5	61.9	64.6
American¶	42.87	45.51	47.83	50.16	51.92	53.94	—	—	—	—	—
Girls											
Rarotongan	40.5	45.0	46.5	48.5	50.0	53.0	55.5	58.5	59.5	60.5	61.5
New Zealand: Maori*	43.25	44.76	47.62	49.78	51.81	54.07	56.53	58.81	60.92	61.63	—
European*	43.45	45.58	48.38	50.05	52.14	54.11	56.70	59.81	61.19	62.63	63.19
English†	—	43.7	45.9	48.0	50.2	52.4	54.5	56.7	58.8	61.0	—
South African‡	—	—	47.3	48.9	51.1	53.6	56.3	58.7	60.7	62.2	63.2
Canadian§	—	44.8	47.1	49.3	51.4	53.4	55.7	58.2	60.2	61.5	62.3
American	—	44.8	47.1	49.2	51.3	53.3	55.6	57.9	60.1	61.6	62.7
American¶	42.52	44.84	47.05	49.17	51.53	53.74	—	—	—	—	—

* Lonie (1945).

† Menzies (1940), quoted by Cluver, Jokl & Rorich (1946) from Benjamin (1943).

‡ Cluver *et al.* (1946).

§ Canadian Government (1942), quoted by Cluver *et al.* (1946).

|| United States Department of Agriculture (1944), quoted by Cluver *et al.* (1946).

¶ Vickers & Stuart (1943).

Table 10. Comparison of average weights (in lb.) of Rarotongan children measured during the survey with those of New Zealand (Maori and European), English, South African, Canadian and American children of similar age

	Age (years)										
	5	6	7	8	9	10	11	12	13	14	15
Boys											
Rarotongan	43.8	44.5	51.8	54.6	63.9	67.9	79.2	84.6	98.5	104.6	123.3
New Zealand: Maori*	45.67	50.20	55.8	62.45	70.08	78.56	83.19	92.40	103.00	118.67	—
European*	43.69	49.19	53.91	60.15	67.01	71.86	75.31	86.85	95.95	108.96	120.14
English†	—	44.9	49.7	54.8	60.3	66.2	72.6	79.4	86.7	94.5	—
South African†	—	46.0	51.1	56.7	63.2	70.0	76.0	83.4	92.7	103.0	115.1
Canadian§	—	46.4	51.3	56.8	62.9	68.8	75.2	82.5	90.8	100.9	111.6
American	—	45.5	50.2	55.5	61.3	67.3	73.6	80.9	90.0	101.0	—
American¶	40.88	45.44	51.81	61.0	68.25	—	—	—	—	—	—
Girls											
Rarotongan	39.9	43.7	48.4	55.0	58.1	70.0	81.0	91.9	102.5	102.5	116.2
New Zealand: Maori*	43.92	46.98	55.74	59.92	68.62	76.48	90.36	101.29	114.34	122.25	—
European*	42.94	47.32	54.32	58.72	64.31	71.16	80.39	87.93	106.91	114.74	118.76
English†	—	43.6	48.1	53.2	59.0	65.6	73.0	81.5	91.2	102.2	—
South African†	—	47.5	49.3	52.4	57.4	64.8	74.8	86.8	98.9	109.5	117.4
Canadian§	—	44.9	49.9	55.0	61.2	67.8	75.8	86.1	95.4	103.4	109.3
American	—	44.1	49.0	54.3	60.1	66.6	74.5	83.9	94.0	102.5	110.2
American¶	41.03	45.12	50.25	55.88	61.01	70.32	—	—	—	—	—

* Lonie (1945).

† Menzies (1940), quoted by Cluver, Jokl & Rorich (1946) from Benjamin (1943).

‡ Cluver *et al.* (1946).

§ Canadian Government (1942), quoted by Cluver *et al.* (1946).

|| United States Department of Agriculture (1944), quoted by Cluver *et al.* (1946).

¶ Vickers & Stuart (1943).

Table 11. *Weight: height (W:H) ratio and Tuxford index (T) of subjects of survey and others calculated from Tables 9 and 10**

		Age (years)										
		5	6	7	8	9	10	11	12	13	14	15
Boys												
Rarotongan:	W:H	1042	1022	1079	1132	1217	1305	1433	1504	1641	1643	1926
	T	1065	999	1007	1006	1027	1044	1082	1069	1094	1017	1113
New Zealand Maori:	W:H	1043	1100	1158	1243	1342	1440	1491	1599	1713	1884	—
	T	1066	1075	1080	1105	1133	1152	1127	1137	1142	1172	—
New Zealand European:	W:H	987	1059	1114	1190	1263	1316	1358	1493	1597	1742	1841
	T	1008	1035	1039	1057	1066	1052	1026	1062	1065	1084	1064
English:	W:H	971	1018	1073	1129	1191	1260	1334	1413	1500	1591	—
	T	992	995	1001	1004	1006	1008	1008	1005	999	989	—
South African:	W:H	—	1000	1064	1134	1215	1296	1357	1438	1545	1653	1782
	T	—	977	993	1008	1026	1037	1025	1023	1029	1028	1029
Canadian:	W:H	—	1028	1082	1145	1217	1284	1357	1437	1528	1637	1746
	T	—	1005	1009	1018	1028	1027	1025	1022	1019	1018	1009
American:	W:H	954	1000	1057	1111	1181	1255	1329	1411	1503	1631	—
	T	975	977	987	988	997	1004	1004	1003	1002	1015	—
Girls												
Rarotongan:	W:H	985	971	1041	1134	1162	1321	1359	1571	1723	1694	1889
	T	1039	975	992	1023	988	1057	1093	1096	1114	1009	1029
New Zealand Maori:	W:H	1015	1049	1170	1204	1324	1414	1598	1722	1877	1984	—
	T	1071	1053	1115	1086	1127	1131	1197	1202	1214	1182	—
New Zealand European:	W:H	988	1038	1123	1173	1233	1315	1418	1470	1747	1832	1879
	T	1043	1042	1070	1058	1049	1052	1062	1026	1129	1091	1023
English:	W:H	—	998	1048	1108	1175	1252	1339	1437	1551	1675	—
	T	—	1002	999	999	999	1002	1003	1003	1003	998	—
South African:	W:H	—	—	1042	1072	1123	1208	1328	1478	1629	1760	1857
	T	—	—	993	967	956	966	995	1031	1054	1049	1011
Canadian:	W:H	—	1002	1059	1116	1191	1269	1361	1479	1584	1681	1754
	T	—	1006	1009	1006	1014	1015	1019	1032	1025	1001	955
American:	W:H	964	995	1056	1122	1177	1249	1340	1449	1564	1663	1757
	T	1017	999	1007	1012	1002	999	1004	1011	1012	991	957

* W:H and T multiplied by 1000 to eliminate decimals.

Table 12. *Mean haemoglobin values determined colorimetrically on the subjects of the survey*

Age (years)	Males		Significance of difference between males and females	Females	
	No. examined	Haemoglobin (g./100 ml. blood)		No. examined	Haemoglobin (g./100 ml. blood)
Under 16	72	13.85 ± 0.19*	Not significant	70	14.25 ± 0.15*
16-45	63	15.29 ± 0.14*	Significant	58	14.30 ± 0.22*
Over 45	21	15.0†	Not significant	20	15.2†
Total	156	14.57 ± 0.12*	Significant	148	13.83 ± 0.12*

* Value with its standard error.
† No. of cases examined considered too small to provide a valid estimate of error.

Table 13. *Mean plasma-protein values determined on subjects of the survey*

Age (years)	No. examined	Plasma protein* (g./100 ml.)	
0-6	29	7.88 ± 0.17	
7-15	110	8.43 ± 0.09	} Difference significant
16 and over	151	8.72 ± 0.08	
Total	290	8.51 ± 0.06	

* Value with its standard error.

Sedimentation rate. The sedimentation rate was measured in 290 people. If normal limits are taken as 5 mm./hr. for men and 15 mm./hr. for women, then the sedimentation rate must be considered abnormally elevated in 67 % of the subjects. In 20 % it was over 30 mm./hr. In three cases it was over 50 mm./hr. In 121 cases with a sedimentation rate of under 15 mm./hr., the mean plasma protein was 8.38 ± 0.082 g./100 ml., whereas in 160 cases with a sedimentation rate of over 15 mm./hr., the mean plasma protein was 8.61 ± 0.72 g./100 ml. This difference shows a probability of 30 : 1 and is taken as significant. The explanation of this observation is considered in the discussion.

Dietary assessment

Questions were asked in each house as to the times when food was eaten and the nature of the food consumed. It was found that, whereas the staple food still remains kumara, taro, breadfruit, arrowroot and green bananas, increasing dependence is being placed on tinned food bought from the stores, of which there were eight in the village of Arorangi alone. About one-third of the total imports to the Cook Island group are foodstuffs, mainly tinned meat, flour, sugar and biscuits. In the *Annual Report* of the Cook Islands for the year ended 31 March 1949, foodstuffs were given as 32.5 % (£97,450) of the total imports (New Zealand Government, 1950). In few families was any milk consumed, and then it was reserved for babies or sick people. Goat's milk was in occasional use. Green vegetables were scarce. Butter, cheese and eggs were rarely eaten. Coconut in some form, particularly as coconut cream, was used in all the families every day. It was surprising to find that fish and fish foods bulked largely in the dietary, despite many statements to the contrary. Each family seemed to collect its own sea-food products. In only one household was flour bought as such.

Milk in schools. The introduction of a milk ration in all the schools in Rarotonga had been in operation for 18 months and the teachers were enthusiastic as to its advantages.

Meals. In every instance breakfast was a very sketchy meal. In some no breakfast at all was eaten. In the remainder it consisted of bread without butter and tea without milk. In only three instances was animal protein eaten at this meal. Frequently the children went to school without breakfast. In all the families, the principal meal of the day was eaten at midday. Fresh meat was a luxury and, when eaten, consisted principally of pork. The evening meal was very similar to the breakfast, consisting chiefly of

bread and tea. Local fruits, such as tomatoes and citrus fruits, were eaten freely when in season, but the pawpaw—rich in vitamin C and a good source of carotene and so attractive in its flavour and texture to the European—was eaten only by babies and pigs.

Vital statistics

Infant-mortality figures have been reported reliably since the introduction of death certification by Cook Islands medical practitioners in 1947. In 1949–50 there were seventy infant deaths and 621 births, giving an infant mortality rate of 113/1000 live births (New Zealand Government, 1950). During 1948–9 only one death from each of marasmus, debility and malnutrition was recorded throughout the whole Cook Islands group (New Zealand Government, 1949). No deaths directly attributable to malnutrition were noted in 1949–50 in Rarotonga. Although deaths in the 1st year of life accounted for 26·3 % of total deaths in 1948–9 and 23·25 % in 1949–50, infections were the greatest cause of death (35 % in the whole islands in 1948–9, and 60 % in Rarotonga in 1949–50). Mortality in the 2nd year of life was 4·5 % of total deaths. Half the population of Rarotonga, which has doubled since 1906, consists of children (Table 1).

DISCUSSION

Nutritional status may be assessed by (a) considering the quantity and quality of foodstuffs consumed, and (b) the results of a clinical examination and laboratory tests designed to discover nutritional disorders.

Davis (1947), Abraham (1947), Peren (1947) and Wingfield (1949) all comment on the change from native to European foods; our findings amply confirm this. While native foods take a minor place, the Rarotongan depends largely upon imported tinned meat, supplemented by fish and shell-fish which are becoming inadequate in supply, for his chief protein sources; all of these are limited by cost or availability. This state of affairs is, in turn, a reflexion on the state of animal husbandry on the island. Very few cows, all in apparently poor condition, are kept; goats and pigs are common domestic animals and most families keep poultry, but there is no attempt at scientific farming either for meat or eggs.

Figures available for infant mortality in both Rarotonga and the whole Cook Islands group, show that infection rather than frank malnutrition is the chief cause of death. How much the high infection rate may be influenced by suboptimal nutrition is not known; infective conditions will be discussed separately in a subsequent publication (Faine & Hercus, 1951).

Protein deficiency, if present, is not reflected either in the plasma proteins or in clinical signs. Indeed, the plasma proteins are relatively high, comparable to figures found for serum by Wills & Bell (1951) in Fiji and Samoa, using the same copper-sulphate method and the same formula for calculation from the specific gravity. A sample of serum submitted to electrophoretic fractionation by these workers showed a twofold increase in the γ -globulin fraction. The increase in plasma protein in Rarotongans is similar, both in magnitude and in age distribution, to the increase in the serums of Fijians and Samoans. Also the association of raised plasma protein

with a rapid sedimentation rate in the Rarotongans suggests that it may be related to some infection.

The results of haemoglobin determinations and of erythrocyte counts showed no signs of iron deficiency, nor were any serious cases of anaemia found, even among women of reproductive age, many of whom had large families.

Clinical examination, substantiated by laboratory findings, showed very little 'malnutrition'. Pollack (1950), in a review of the physical signs of nutritional disturbance, points out that the degree of abnormality required for the diagnosis of nutritional disturbance varies with the observer and with his experience of nutritional disorders. Nevertheless, none of the signs he considers indicative or suspicious of nutritional disturbance were seen, so that the problem of the interpretation of signs noted does not arise. A clinical or laboratory search for signs of malnutrition is, at the best, a negative approach to the problem of assessment of nutritional status. It is more important to be able to credit for 'good' nutrition than to debit for 'malnutrition'. The 'subjective nutritional assessment' contributes to the positive side to some extent. Here the difficulty is not deduction of marks for 'malnutrition' but how to assess obesity, which was common. If obesity is to be considered a state of nutritional disturbance, as it is, then marks must be deducted; if this is done there is no way of distinguishing between the obese and the undernourished. However, if this distinction is not made, there is still a difference between those considered to be of good nutrition and those of unsatisfactory nutrition, whether the latter state be due to undernourishment or obesity. Here marks have been deducted for obesity.

Obesity, as seen from the height-weight graph, is an important problem, especially among the women, very few of whom are tall in proportion to their weights. A dietary factor that alone could be responsible was not obvious, unless it could be the apparent reliance placed on foods in the diet containing carbohydrate and fat. Diabetes is said to be rare. Perhaps there is a racial factor, possibly of dietary habit, as this obesity appears to be common to several Polynesian groups, especially among the women. Many workers have attempted to provide some index, readily arrived at from a few simple measurements, that would give an objective measurement of nutritional status. Of nearly all of these methods, reviewed by Tuxford (1939), Stuart & Meredith (1946) and Stevenson (1950), height and weight are the fundamental factors, which are corrected for age by some when dealing with children. There is little doubt that physique reflects nutritional disturbances, so that measurement of physique may be taken as a measure of a positive aspect of nutritional status, in the absence of gross organic factors affecting physique. Height and weight are both gained progressively during childhood and adolescence, but there is a wide range of normal values in each age group due to individual variations in body-type, sexual maturity and racial factors. For these reasons ratios or indices based on height and weight have been recommended as a more constant measure of the individual's physique, irrespective of age. On the other hand, since the ratio of weight to height varies with age in normal children, it seems desirable that age be taken into consideration. Attempts have been made to standardize this factor in various ways. Tuxford (1939, 1942) has recommended the use of a formula which reduces the curved $W:H$ graph to a straight line, along an 'index'

of 1000 in London schoolchildren. Wetzel (1941), modifying the $W:H$ ratio for age and body type, has developed an index which is excellent for following individual cases in successive examinations, but is of little significance for single observations, as in this survey. Here we have preferred to use the Tuxford index, which means that a child with a higher index is heavier for its height, than other comparable children, regardless of age. It will be seen from Fig. 2 that the Tuxford index is approximately 1000 only for English, North American and Canadian children, whereas Rarotongan, South African and New Zealand children, both Maori and European, vary considerably from this level. Recently published figures show that with an increase in average heights and weights in London children (Daley, 1950), there was only a slight increase in Tuxford's index, especially for girls, since 1938.

These considerations emphasize the reflexion of physique in the Tuxford-index score, and the value of some such ratio for providing a base-line for comparison between groups. It is obvious, however, that a different manipulation of the $W:H$ ratio for age must be made to allow for racial or local variations, as with New Zealand Maoris, for example, to give an index of 1000. It will also be noted from Fig. 2 that an endocrine factor probably plays some part in determining the differences between groups, as the variation from 1000 is most marked in each group among girls from 10 to 15 years old.

The results show that the physique of Rarotongan children and adults compares favourably with that of people in other parts of the world, and that it does not reflect signs of malnutrition.

Comparison of the heights and weights of Rarotongan children with figures for children of other countries (Meredith, 1948; Barach, 1945) substantiates this.

Dental conditions in Rarotonga have been reported by Wingfield (1949), who found that the incidence of dental caries in schoolchildren averaged eight teeth per child, two-thirds of the cavities being in the early stages of decay (stages 1 and 2) and one-third in advanced stages (stages 3 and 4). An examination of 500 adults showed also eight carious teeth per head, and abnormal gum conditions in 48 %. The findings in this survey show an almost universal incidence of caries with some increase in degree with age. Also, there is a steady increase with age in the number of teeth missing and a general picture of absence of prevention or treatment of dental disease. The only people with teeth filled are those who are young enough to have come under the influence of the School Dental Service set up a few years ago; it is from this end that the problem is being tackled. The state of the gums, though most unsatisfactory, compares favourably with the findings in Samoa by the 1948-9 expedition (unpublished report to New Zealand Medical Research Council). To what extent the hypoplasia of the enamel found in some cases, or the large amount of caries and gingivitis, comes from a nutritional background, is difficult to tell. There is no evidence for or against calcium deficiency, which is not believed to be present. There is almost certainly a relationship to the introduction of European foods, as it has been recorded by several observers that the natives' teeth were in excellent condition many years ago. Also, inhabitants of Rarotonga, some of them among the group studied, tell how their teeth were free from dental caries on other islands where they lived until they moved to Rarotonga;

here they consumed a much larger amount of European food, especially soft carbohydrates, whereas on the other Cook islands they had to exist on hard native foods.

In the absence of other signs of nutritional deficiency, which might be expected if these oral changes found represented a lack of essential dietary components, it might be concluded that the problem is primarily an oral one, and could be remedied by education in oral hygiene, efficient prophylaxis and treatment in the early stages of dental caries.

It was of considerable interest to note that there was only one mild case of thyroid enlargement; the large quantity of fish and sea-foods consumed must provide a relatively large iodine intake.

From all these findings, it will be seen that the diet must essentially be adequate in quality and quantity. Inasmuch as the economic status of the individual governs the amount and type of food he consumes, so long as he relies (especially for animal protein) upon imported food that must be bought, the diet could be improved by better planning of local food production and by the fostering of animal and poultry husbandry.

SUMMARY

1. A representative sample of Rarotongans has been investigated by clinical and laboratory methods and their nutritional status assessed.
2. The results showed that malnutrition is neither a widespread nor a serious problem except that the influence of European food apparently plays an important part in the very common unhealthy state of the teeth and mouth.
3. The diets were apparently sufficient in protein, carbohydrate, fat, minerals and vitamins.
4. Obesity was common, due possibly to racial as well as dietary factors. The physique of both adults and children compared favourably with that of similar groups in other countries.

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The Nutritive Value of Colostrum for the Calf

5. The Effect of Prepartum Milking

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The milking of cows and heifers before parturition has been advocated as a means of reducing the congestion of the udder and the associated oedema at calving, and of increasing the milk yield of the cow during the subsequent lactation period. The extent of pre-milking varies widely; on some farms, cows are milked only once or twice before parturition, whereas on others they are milked twice daily for up to 30 days.

The composition of the secretion produced at calving is altered by prepartum milking (Eaton, Johnson, Spielman, Matterson & Nezvesky, 1949*a*), there being a reduction in the total nitrogen and in the percentage of non-casein nitrogen; the extent of this decline depends on the quantity of secretion removed before calving (VanLandingham, Weakley, Ackerman & Hyatt, 1949). It is possible that the postpartum secretion of pre-milked cows may be of less benefit to newborn calves than normal colostrum, and it

was observed in America that calves from dams that had been milked prepartum were more difficult to rear (Turner 1931, Davis & Trimberger, 1941). Furthermore, calves given for 1 week fixed amounts of the milk from their pre-milked dams gained less weight than calves receiving colostrum (Eaton, Johnson, Spielman, Matterson & Nezvesky, 1949*b*). The gain in weight of the calves improved if vitamin A was given to the dams for 30 days before calving, but even so the gains were lower than those of calves receiving normal colostrum. The differences were not, however, statistically significant, but they may have been masked by the effect of sodium sulphamethazine given as a therapeutic agent when scouring began. Keyes, Reid, Bechdel, Borland, Beam & Williams (1944) found that only four of the calves of twenty-five cows pre-milked for 2-16 days scoured, and these recovered within 7 days; during this time a carotene preparation was given daily. Ackerman, Hyatt & VanLandingham (1949) record the performance of calves from twenty-four pre-milked Ayrshire cows; these grew normally without scouring when given cod-liver oil for the first 3 days. Only nine of these cows gave over 20 lb. of secretion on the day preceding parturition. The authors consider that only such cows give at calving a secretion of the composition of normal milk.

Hill, Widdowson & Maggs (1950) reported the results of a preliminary investigation under practical conditions, and concluded that pre-milking was not detrimental to the health of either cow or calf. It appears from their paper that pre-milking was more often than not restricted to 2 or 3 days before calving, and it therefore seems likely that most of the calves received secretions that differed but little from normal colostrum.

To obtain further evidence on the effect of prepartum milking on the health and performance of the newborn calf, equal volumes of either colostrum or of the postpartum secretion of pre-milked cows were given to calves during the first 24 hr. of life. For comparison, the effect of giving the same volume of ordinary milk was also studied.

METHODS

Experimental layout

This experiment was done in the autumn of 1949. A randomized block design was used, with three treatments in each of six blocks of calves, as follows:

Treatment	
No.*	Initial diet
10	Colostrum
11	Milk
12	Postpartum secretion of pre-milked cows.

* The numbers are consecutive to the numbers given to the treatments in previous experiments.

Diets

Basic diet

The calves received one of the initial diets listed below during the first 24 hr. of life, and were then reared for 3 weeks on 'synthetic milk' (Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin & Lovell, 1949). The maximum daily allowance was 1 lb./10 lb. live weight except when scouring occurred (see below).

Initial diets

Colostrum was collected during the first 24 hr. after parturition from each of five Shorthorn heifers in the N.I.R.D. herd; 1-pint samples were stored separately at -25° . Each calf on treatment 10 was given 5 pints of colostrum consisting of one pint from each heifer.

Milk. The afternoon and morning milk given by the same five heifers 14 days after calving was stored at -25° . It was blended and given to calves on treatment 11 in the same way and quantity as colostrum in treatment 10.

Postpartum secretion of pre-milked cows. Eleven Shorthorn cows from the N.I.R.D. herd were milked by hand twice daily starting on the 14th day before calving was expected; the mean period of pre-milking with its standard error was 16 ± 1 days. The cows were 'steamed up' for a mean period of 37 ± 1 days before calving with a compound concentrate (National Cattle Food no. 1) starting at 4 lb. daily, and gradually increasing to 8 lb. daily for the last fortnight. The total amount secreted by the eleven cows before calving varied from 1 to 96 l. The variability in total yield agrees with that observed by VanLandingham *et al.* (1949) who found total yields varying from 2 to 142 lb. for eight first-calf Ayrshire heifers pre-milked for 10 days. They suggested that prepartum milking might stimulate the production of lactogenic hormone and pointed out that cows vary in their response to this stimulus.

The secretions to be fed to the experimental calves were collected in the first 24 hr. after parturition from five of the pre-milked cows and were stored separately at -25° . These five cows were chosen as they had responded well to pre-milking by giving a mean total yield before parturition of 50.7 ± 5.3 l. and a mean yield of 12.8 ± 2.3 l. on the day before calving. The secretions were blended and given to calves on treatment 12 in the same way and quantity as with colostrum in treatment 10. All pre-milked cows calved normally, except one not used in this experiment which everted her uterus and developed milk fever.

Composition. Samples of rennet whey were prepared from every individual secretion used in blending the initial diets of the calves. The total-nitrogen values of the whey samples are given in Table 1.

Table 1. Total nitrogen (g./100 ml.) of whey samples prepared from secretions used for initial feeding of calves

Batch no.	Treatment (no. and initial diet)		
	10 Colostrum	11 Milk	12 Postpartum secretion of pre-milked cows
1	0.794	0.144	0.228
2	2.209	0.152	0.217
3	0.839	0.149	0.233
4	2.145	0.147	0.215
5	1.633	0.148	0.192
Mean	1.524	0.148	0.217

The nitrogen content of the secretions of pre-milked cows obtained during the first 24 hr. after parturition was remarkably uniform. Though higher than that of milk, it was greatly reduced in comparison with that of normal colostrum. As equal volumes of the initial diets were given to the calves, those on treatments 11 and 12 received far less, if any, of the 'immune lactoglobulins' present in the whey of normal colostrum.

The whey samples for which data are listed in Table 1, together with whey samples prepared from the secretions produced on the 1st and 7th days of prepartum milking by the five cows whose secretions were used for treatment 12, were tested for agglutinins against the five strains of *Bacterium coli*, isolated from the calves that died on this experiment. This aspect of our work will be discussed by Briggs in a forthcoming paper of this series (Briggs, 1951). There was a decline in activity during the period of prepartum milking and no agglutinins could be demonstrated in the secretions obtained during the first 24 hr. after calving. Similarly, the samples of milk collected 14 days after calving had no activity, except in one instance in which the corresponding colostrum had an exceptionally high titre.

Calves

Shorthorn bull calves were collected and managed as in earlier experiments (Aschaffenburg *et al.* 1949). On the arrival of a newborn calf, a blood sample was taken and the globulin-turbidity test (Aschaffenburg, 1949) was applied to the serum to verify that the calf had not suckled. Calves were rejected if the test was positive.

The calves were fed three times daily for the first 10 days of life, and twice daily for the remainder of the experimental period of 3 weeks. If a calf scoured, it was given water, warmed to 37°, at subsequent feeds. When scouring no longer occurred, synthetic milk diluted with an equal amount of water was given, and the proportion of milk was then gradually increased at each feed until the 'synthetic milk' alone was again used. This procedure was repeated when scouring recurred. Records were kept as in the earlier experiments (Aschaffenburg *et al.* 1949).

RESULTS

Performance of calves

The results for the six blocks of calves are summarized in Table 2.

It is evident that treatments 11 and 12 were inferior to treatment 10; all calves given normal colostrum survived, whereas two calves on treatment 11 and three on treatment 12 died. The differences in the number of deaths between the three treatments were not, however, significant ($P < 0.2$ between treatments 10 and 12 and $P < 0.5$ between treatments 10 and 11).

Before comparing the gains in weight and frequency of scouring for the calves on the three treatments, the residual error due to differences between the performance of calves on each block was taken out, the 'missing plot technique' of Yates (1933) being used to calculate values for the calves that died. Analysis of covariance of live-weight gain on birth weight, and analysis of variance of the number of days on which scouring occurred (x), with values transformed $\sqrt{(x + \frac{1}{2})}$ were made.

Table 2. Performance of calves given colostrum, milk or the postpartum secretion of pre-milked cows

	Treatment (no. and diet given)		
	10 Colostrum	11 Milk	12 Postpartum secretion of pre-milked cows
Calves:			
No. used	6	6	6
No. died	0	2	3
Age of calves at death (days) and autopsy finding	—	11*, 14†	5*, 9†, 11*
Adjusted mean live-weight gain of calves during:			
7 days (lb.)‡	+1 ± 1.2	-1 ± 1.4	-7 ± 1.6
14 days (lb.)‡	+2 ± 0.8	-6 ± 0.9	-8 ± 1.1
21 days (lb.)‡	+8 ± 2.4	-7 ± 2.9	-4 ± 3.3
Mean no. of days on which surviving calves scoured‡	3 ± 0.4	6 ± 2.5	8 ± 1.2

* *Bact. coli* septicaemia.† *Bact. coli* peritonitis and pleurisy.

‡ Values with their standard errors.

The relation between live-weight gain and birth weight of the calves was significant at 14 and 21 days ($P < 0.01$) but not at 7 days. The regression equations were:

Live-weight gain during 7 days (lb.)

$$= -2.57 + 0.08 (\text{birth weight} - 87.26) \quad \text{S.E.} = 2.8.$$

Live-weight gain during 14 days (lb.)

$$= -4.11 + 1.04 (\text{birth weight} - 87.26) \quad \text{S.E.} = 5.7.$$

Live-weight gain during 21 days (lb.)

$$= -0.85 + 1.76 (\text{birth weight} - 87.26) \quad \text{S.E.} = 1.9.$$

There were no significant differences between the number of days on which calves scoured on the three treatments, nor between the adjusted mean live-weight gains during 7, 14 and 21 days for calves on treatments 11 and 12.

Differences were, however, significant between adjusted mean live-weight gains during 14 and 21 days (both $P < 0.05$) for calves on treatments 10 and 11, and during 14 days ($P < 0.01$) and 21 days ($P < 0.05$) for calves on treatments 10 and 12. No significant differences were found between adjusted mean live-weight gains during 7 days for calves on treatments 10 and 12.

Autopsy findings

A summary of the autopsy findings is given in Table 2 which shows that all five calves died from a *Bact. coli* infection. Three of the five calves had a *Bact. coli* septicaemia and two died from *Bact. coli* peritonitis and pleurisy.

These two manifestations of 'colibacillosis' may indicate a variation in the resistance of the host but they are basically the same disease; *Bact. coli* is widely distributed throughout the tissues in both.

DISCUSSION

The findings show that, on a volume basis, the secretion produced in the first 24 hr. after calving by cows that had responded well to pre-milking for a mean period of 16 ± 2 days was, like milk, of less value for the well-being of the newborn calf than normal colostrum.

The results of giving to calves the postpartum secretion from pre-milked cows were no better than those of giving ordinary heifer milk collected 14 days after calving. The relatively poor performance on both these treatments was in keeping with the reduced intake of colostral globulins, and resembles that of calves that, in earlier experiments (Aschaffenburg, Bartlett, Kon, Roy, Walker, Briggs & Lovell, 1951) received small quantities of 'immune lactoglobulins'. The poorer performance is also linked with the decline in the agglutinin content of the secretions, and it is doubtful whether newborn calves, even if given larger volumes of the milk-like postpartum secretion of the pre-milked animals used in our experiments, would get as good a start in life as those receiving normal colostrum. This doubt would hardly arise where, as often happens under practical farming conditions, pre-milking is restricted to the relief of congestion of the udder on the last day or two before calving, since the calf would then receive postpartum secretions differing but little from normal colostrum. It is also possible that the calves used in this experiment were exposed to a greater risk of infection, owing to the presence in the calf pens of susceptible calves from many different farms, than is likely to occur under normal farm conditions.

SUMMARY

1. Eighteen newborn Shorthorn bull calves, grouped in six blocks, were given normal colostrum, milk, or the secretion produced after parturition by cows pre-milked for 14 days. These diets were fed during the first 24 hr. of life and thereafter the calves were reared for 3 weeks on a standard diet based on dried skim milk.
2. All calves given normal colostrum survived, whereas three given the secretion after prepartum milking, and two given milk, died.
3. There were no significant differences between the frequencies of scouring of calves on the three treatments, or between the mean live-weight gains of calves given milk and the secretion produced at parturition after prepartum milking. The mean live-weight gain at 14 and 21 days, adjusted for birth weight, of calves that had received normal colostrum was significantly greater than that of calves on the other two treatments.

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The Nutritive Value of Colostrum for the Calf

6. The 'K' Antigens of *Bacterium coli**

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Smith & Little (1922) drew attention to the protective properties of colostrum against white scours of calves. Since 1946 members of the staffs of the Department of Pathology of the Royal Veterinary College and of the National Institute for Research in Dairying, University of Reading, have collaborated in experiments planned to assess the relative importance of the nutritional and the immunological properties of colostrum for the calf, and have published their findings in several papers (Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin & Lovell, 1949; Aschaffenburg, Bartlett, Kon, Walker, Briggs, Cotchin & Lovell, 1949; Aschaffenburg, Bartlett, Kon, Roy, Walker, Briggs & Lovell, 1951). These workers showed that small quantities of colostrum, of its aqueous fraction and of globulin constituents, protected calves against white scours; the indication was that the mechanism of such protection might be of an immunological nature. The work reported in the

* This work formed part of a thesis submitted to the University of Reading for the degree of Ph.D., and the substance of it was read at a meeting of the Pathological Society on 6 January 1950.

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present paper was undertaken to provide a basis for an investigation of the immunological aspects of the protective action of colostrum. The results of that investigation are recorded in a subsequent paper (Briggs, Lovell, Aschaffenburg, Bartlett, Kon, Roy, Thompson & Walker, 1951).

White scours is frequently associated with *Bacterium coli* infection, and several workers believe that certain races of *Bact. coli* are potentially pathogenic for calves. Jensen (1892-3), Smith & Little (1922) and Smith & Orcutt (1925) based their conclusion largely on the classification of strains by biochemical reactions. Lovell (1937) reached a similar conclusion by classification on serological grounds. His precipitin tests with serums and extracts of strains of *Bact. coli* that showed grey-mucoid variation gave unequivocal results, but agglutination tests indicated the presence of two separate antigens apart from a flagellar one; these were associated with the body of the bacterium and the capsule respectively.

The antigens of *Bact. coli* have been studied by Knipschildt (1945), Vahlne (1945) and Kauffmann (1947), and a serological classification of the *coli* group has been established. Apart from the 'O' or somatic antigens there are two main 'K' or 'capsular' antigens, the 'A' antigens and the 'L' (or 'B') antigens. 'A' antigens are thermostable and responsible for 'O'-inagglutinability of suspensions of living bacteria and of those heated at 100° for 1 hr.; they are destroyed by heating to 120° for 2 hr. 'A' strains produce mucoid colonies of capsulated bacteria. 'L' (or 'B') antigens are thermolabile and responsible for 'O'-inagglutinability of suspensions of living bacteria; they are destroyed by heating to 100° for 1 hr. 'L' strains produce grey colonies of non-capsulated bacteria. 'B' antigens closely resemble 'L' antigens but are very rare.

Kauffman (1947) considers that it is possible to differentiate between 'L' and 'A' antigens by the determination of 'O'-inagglutinability and that the rare 'B' antigens can be largely ignored. In the light of these observations a study has been made of the 'K' antigens of *Bact. coli* isolated from calves to determine their significance in the pathogenesis of white scours.

METHODS

Source of strains. The calves that died of white scours in the experiments of Aschaffenburg, Bartlett, Kon, Terry *et al.* (1949) and Aschaffenburg, Bartlett, Kon, Walker *et al.* (1949) constituted the main source of the strains of *Bact. coli* studied in this investigation. Six cases of naturally occurring white scours were also obtained for purposes of comparison. The majority of the strains were recovered from the heart blood and bone marrow of the calves.

Cultivation of strains. Cultures were made initially on blood agar and McConkey agar plates, after which a preliminary identification of the strains was made, based on their biochemical reactions. The strains were preserved in wax-sealed stabs of 0.5 % agar at room temperature in the dark, and later by freeze-drying.

Immune serums. These were prepared in rabbits by a series of injections of 0.25 % formalinized suspensions of mucoid or grey cultures. The strains were from representative experimental calves and the relevant data are given on the next page.

Serum reference	No. of calf and of strain	Type of strain	Type of colony*	Presence of capsules (microscopic examination)†
RR	28	<i>Bact. coli</i> intermediate type II	M	+(-)
H	4	<i>Bact. aerogenes</i>	M	+(-)
JJ	23	<i>Bact. coli</i> type I	M	+(-)
TT	29	<i>Bact. coli</i> type I	M	+(-)
LLL	2 A	<i>Bact. coli</i> type I	G	-

* M = mucoid; G = grey.

† (-) = a very few organisms did not show capsules.

Precipitin tests were made by mixing immune serum with alkali extracts of strains as prepared by Smith (1927).

Agglutination tests. Three suspensions were prepared for differentiating between 'L', 'A' and 'O' agglutination:

(1) Living suspensions prepared from 20 hr. growth on thick, dry, plain agar; this largely suppresses the development of flagellar antigens; at the same time it promotes the development of 'K' antigens (Kauffmann, 1947).

(2) The same suspensions heated at 100° for 1 hr.

(3) Living suspensions heated at 120° for 2 hr.

The serum dilutions used were 1:10-1:5120, and the tubes were incubated at 37° for 18 hr.

Agglutinin-absorption tests. Absorption of serums with thick suspensions of bacteria was made at 4° overnight; the absorbed supernatant fluid was tested against the same bacterial suspensions that were used in the agglutination tests.

Mouse-protection tests. For each strain tested the quantity of a 20 hr. broth culture that, after intraperitoneal injection, killed about half the mice injected was calculated, and was taken as the LD₅₀ dose: this was mostly about 0.12 ml. Mice were injected intraperitoneally with 0.5 ml. of a 1:5-1:20 dilution of serum, depending on the agglutinin content of the sample; one LD₅₀ of the test strain was similarly given 2 hr. later. The mice were observed for 72 hr. after inoculation, since in preliminary trials no deaths occurred later than this.

RESULTS

Biochemical types of strain. On the basis of biochemical tests most strains were classified as *Bact. coli* type I: a few strains of *Bact. coli* intermediate type II and of *Bact. aerogenes* were also recovered from cases of white scours.

Precipitin tests. Sixty-two strains were submitted to the precipitin test against each of the five samples of immune serum listed above: forty-nine (79 %) reacted with one or other of the serums, as shown below, thirteen strains remaining unclassified.

No. of strains	Un-classified	Reacting with serum					Total
		RR	H	JJ	TT	LLL	
	13	5	4	12	22	6	62

Agglutination tests. Agglutination tests were made with 102 strains of colibacteria and the relationship between the precipitin reaction and the agglutination of a particular

suspension was noted. Some of the strains examined were recovered from the same calf. In general, those labelled (a) (e.g. 32(a)) were from the blood and bone marrow, and presumably had invasive characteristics. Those labelled (b) (e.g. 32(b)) were mostly from the intestinal tract or mesenteric lymph nodes. The results for twenty strains are given in Table 1; information concerning haemolytic activity and the type of colony is included.

The interpretation of the agglutination results is based on the reactions obtained with the different suspensions. For example, the agglutination by a particular serum of living suspensions and of those heated to 100° but not of those heated to 120° suggests that the agglutination observed was due to the presence of the thermostable 'A' antigen and its corresponding antibody. The 'L' antigen is thermolabile and its inhibitory effect on 'O' agglutination disappears when suspensions are heated at 100° for 1 hr. The results obtained with strains 3A and 28 are examples of 'A' agglutination and those with strains 17 and 19 of 'L' (or 'B') agglutination.

When one serum agglutinates all three suspensions of a strain to approximately similar titres, agglutinin-absorption tests are necessary in order to distinguish between 'K' ('capsular') and 'O' (somatic) agglutination reactions. In some instances with suspensions heated to 120°, agglutinins against the 'O' antigen were in this way completely removed, whereas those against living suspensions and suspensions heated to 100° were left intact: this demonstrated 'A' agglutination. In other instances, absorption with similarly treated suspensions removed agglutinins also against suspensions heated to 100°, thereby indicating that the 'L' antigen and its antibody were involved. Where a suspension heated to 120° proved capable of preventing agglutination by a serum of living and heated suspensions of the strain it was concluded that the test strain did not contain the relevant 'K' antigen, and that the agglutination was in all instances due to the 'O' antibody of the serum.

The relationship between the results obtained by the precipitin technique and the agglutination of the 'K' antigens (either 'L' or 'A') will be discussed later.

Mouse-protection tests. It is impracticable to give here full details of the numerous mouse-protection tests made; they all gave similar results, and typical examples are given in Table 2. The separate total mortality figures for mice receiving serums (a) with 'K', (b) with ('O'), and (c) without 'K' or 'O' antibodies were 3/60, 31/80 and 20/40 respectively. These values were examined by the χ^2 test, incorporating Yates's (1934) correction for continuity; no significant difference at the 5 % level was found between the results of treatments (b) and (c), but the effects of treatment (a) proved highly significantly ($P < 0.001$) different from those of either (b) or (c). These results showed that serums with the relevant 'K' antibody ('L' or 'A' agglutination) conferred a high order of protection, whereas serums lacking 'K' antibody, whether or not containing 'O' antibody ('O' agglutination or no agglutination), conferred no protection. It should be remembered that the test dose of organisms was calculated to kill half the mice inoculated.

Table 1. *Results of precipitation and agglutination tests with twenty strains of Bact. coli recovered from cases of white scours*

No. of calf and of strain, and type	Type of colony	Serological tests						Interpretation of results	
		Haemolysis	Antigenic substance	Serums					
RR	H			JJ	TT	LLL			
17, <i>Bact. coli</i> I	G	—	Ext. Susp. 1 2 3	— — 320 640	— — — —	+ 1280 — —	— — 640 1280 1280	— — — — —	Precipitation 'L' agglutination 'O' agglutination
19, <i>Bact. coli</i> I	G	—	Ext. Susp. 1 2 3	— — — —	— — — —	— — — —	— — 1280 1280	+ 640 — —	Precipitation 'L' agglutination 'O' agglutination
8, <i>Bact. coli</i> I	G	—	Ext. Susp. 1 2 3	— — — —	— — — —	+ 1280 — —	— — — —	— — — —	Precipitation 'L' agglutination
30, <i>Bact. coli</i> I	G	+	Ext. Susp. 1 2 3	— — — 160	— — — —	— — — —	— — 640 1280	+ 2560 — —	Precipitation 'L' agglutination 'O' agglutination
3A, <i>Bact. coli</i> I	G	—	Ext. Susp. 1 2 3	— 80 80 2560	— — — —	— — — —	+ 1280 640 —	— — — —	Precipitation 'A' agglutination 'O' agglutination
8A, <i>Bact. coli</i> I	G	—	Ext. Susp. 1 2 3	— — — —	— — — —	+ 1280 — —	— — 1280 2560	— — — —	Precipitation 'L' agglutination 'O' agglutination
12, <i>Bact. coli</i> I	G	—	Ext. Susp. 1 2 3	— — — —	— — — —	+ 2560 — —	— — — —	— — 320 640	Precipitation 'L' agglutination 'O' agglutination
22, <i>Bact. coli</i> I	G	—	Ext. Susp. 1 2 3	— — — —	— — 160 80	— — — —	— — 2560 2560	+ 640 — —	Precipitation 'L' agglutination 'O' agglutination
5A, <i>Bact. coli</i> I	G	—	Ext. Susp. 1 2 3	— — — —	— — — —	+ 1280 — —	— — 2560 2560	— — — —	Precipitation 'L' agglutination 'O' agglutination
28, <i>Bact. coli</i> I	M	—	Ext. Susp. 1 2 3	— — — 160	— — — —	— — — —	+ 2560 2560 —	— — — —	Precipitation 'A' agglutination 'O' agglutination
32(a), <i>Bact. coli</i> I	M	—	Ext. Susp. 1 2 3	— — — —	— — — —	— — — —	+ 2560 2560 —	80 160 1280	Precipitation 'A' agglutination 'O' agglutination
32(b), <i>Bact. coli</i> I	G	+	Ext. Susp. 1 2 3	— — — 160	— — — —	— — — —	+ 640 640 1280	— — — —	Precipitation 'A' agglutination 'O' agglutination
33(a), <i>Bact. coli</i> I	G	—	Ext. Susp. 1 2 3	— — — —	— — — —	— — — —	+ 1280 1280 —	80 80 2560	Precipitation 'A' agglutination 'O' agglutination
33(b), <i>Bact. coli</i> I	M	+	Ext. Susp. 1 2 3	— — — 160	— — — —	— — — —	+ 640 1280 1280	— — — —	Precipitation 'A' agglutination 'O' agglutination
34(a), <i>Bact. coli</i> I	G	—	Ext. Susp. 1 2 3	— — — —	— — — —	— — — —	+ 1280 1280 —	160 2560	Precipitation 'A' agglutination 'O' agglutination
34(b), <i>Bact. coli</i> I	M	+	Ext. Susp. 1 2 3	— — — 80	— — — —	— — — —	+ 640 2560 2560	— — — —	Precipitation 'A' agglutination 'O' agglutination
35(a), <i>Bact. coli</i> I	G	—	Ext. Susp. 1 2 3	— — — —	— — — —	640 — — —	— 1280 2560	— — — —	Precipitation 'L' agglutination 'O' agglutination
35(b), <i>Bact. coli</i> I	G	+	Ext. Susp. 1 2 3	— — — 80	— — — —	— — — —	+ 1280 1280 1280	— — — —	Precipitation 'A' agglutination 'O' agglutination
4A, <i>Bact. coli</i> intermediate type II	G	+	Ext. Susp. 1 2 3	— — — —	— — — —	— — — —	+ 1280 1280 —	— — — —	Precipitation 'A' agglutination
14, <i>Bact. coli</i> intermediate type I	G	+	Ext. Susp. 1 2 3	— — — —	— — — —	— — — —	+ 640 1280 —	— — — —	Precipitation 'A' agglutination

G = grey; M = mucoid. Ext. = alkali-alcohol extract for precipitin test. Susp. = suspensions for agglutination tests: 1, living; 2, heated, 100° for 1 hr.; 3, heated, 120° for 2 hr. The results of the precipitin tests are expressed as + or —. The titres in the agglutination tests are expressed as the reciprocals of the dilutions; — = no agglutination at 1:10.

Table 2. *Relationship between mouse-protection tests and serological tests in vitro*

Strain no.	Test	Serums				
		RR	H	JJ	TT	LLL
5 A	Precipitation	—	—	+	—	—
	'L' agglutination	—	—	1280	—	—
	'O' agglutination	—	—	—	2560	—
	Mouse mortality	N.T.	N.T.	1/20	6/20	10/20
12	Precipitation	—	—	+	—	—
	'L' agglutination	—	—	2560	—	—
	'O' agglutination	—	—	—	—	640
	Mouse mortality	N.T.	N.T.	2/20	12/20	4/20
28	Precipitation	—	—	—	+	—
	'A' agglutination	—	—	—	2560	—
	'O' agglutination	160	—	—	—	—
	Mouse mortality	9/20	N.T.	N.T.	0/20	10/20

The results of the precipitin tests are expressed as + or —. The titres in the agglutination tests are expressed as the reciprocals of the dilutions. Each mouse received one LD₅₀ dose of the test organism. The mouse-mortality figures give the mortality in the numerator and the number inoculated as the denominator. — = no agglutination at 1:10; N.T. = not tested.

DISCUSSION

The results show a close relationship between the precipitin reaction and the agglutination of the 'K' antigen of a strain of *Bact. coli*. A similar observation has been recorded by Giles & Sangster (1948) who worked with strains of *Bact. coli* recovered from cases of infantile diarrhoea. Some of their tests were made with strains producing grey colonies (see Lovell, 1937), and the 'K' antigen involved was probably the 'L' type as distinct from the capsular 'A'. The results of protection tests in mice showed that a serum containing the precipitin and the antibody against the 'K' antigen of a given strain of *Bact. coli* protected mice infected with that strain.

Precipitation and agglutination tests permit a classification of colibacteria; they are of value in epidemiological inquiries and indicate the probable spread of strains of *Bact. coli* from calf to calf during an epidemic of white scours. For example, the non-haemolytic *Bact. coli* strains, nos. 32(a), 33(a) and 34(a), were recovered from calves that lived in the same environment and died within a few days of each other: these strains possessed similar antigens and were presumably one and the same strain. Similarly, a series of haemolytic strains, 32(b), 33(b), 34(b) and 35(b), was recovered from the same group of calves and these too were serologically identical. The passage of strains from calf to calf in an enclosed community was thus demonstrated. In random cases of white scours occurring in the field, conditions are different; strains recovered from such cases were found to be serologically unrelated. These observations suggest that outbreaks of white scours are associated with the multiplication of special races of *Bact. coli*, which develop their pathogenicity under suitable conditions.

SUMMARY

1. Strains of *Bact. coli* were recovered from calves that had suffered from white scours. Serological examination provided evidence that the 'K' antigens and the pathogenic activities of strains were related.

2. There was a close relationship between the results of 'K' antigen-antibody agglutination tests, precipitin tests using as antigens alkali extracts of strains, and protection tests in mice.

3. Serological classification of coliform organisms demonstrated the spread of potentially pathogenic strains from calf to calf during epidemics of white scours.

I wish to record my gratitude to Dr R. Lovell for his unfailing help and invaluable guidance. My thanks are also due to Dr T. Richards, and the members of the National Institute for Research in Dairying who are collaborating in these studies.

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The Nutritive Value of Colostrum for the Calf

7. Observations on the Nature of the Protective Properties of Colostrum

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The mechanism of the protective action of colostrum in white scours of calves is not understood. Smith & Little (1922) recognized the protective value of the secretion, and Little & Orcutt (1922) demonstrated the passage of agglutinins for *Brucella abortus* from cow to calf in colostrum. Nelson (1924) found a similar transfer of agglutinins for *Bacterium coli* but there was no evidence that these agglutinins were linked with protection. Smith (1930) established that protective antibodies for *Bact. coli* were present in colostrum and that the low content of certain samples was related to the recent entry of a cow into her particular environment; he assumed that such cows had not yet become immunized against the new flora.

The recent work on the antigenic analysis of *Bact. coli* and the evidence that the 'K' antigen-antibody reaction is linked with protection (Briggs, 1951) provided a basis for an immunological study of the protective value of colostrum. This study was made in conjunction with experiments in which newborn calves received various fractions of colostrum as reported in earlier papers of this series (Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin & Lovell, 1949; Aschaffenburg, Bartlett, Kon, Walker, Briggs, Cotchin & Lovell, 1949; Aschaffenburg, Bartlett, Kon, Roy, Walker, Briggs & Lovell, 1951*a, b*). The essential protective factor against white scours appears to be associated with the lactoglobulin fraction (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949; Aschaffenburg, Bartlett, Kon, Walker *et al.* 1949; Aschaffenburg *et al.* 1951*a*).

The object of this paper is to show that samples of colostrum may contain antibodies against the 'K' antigens of *Bact. coli* and that these antibodies, as in immune serum, are linked with protection.

METHODS

The techniques adopted were similar to those used in the tests with immune serum (see Briggs, 1951), serum being replaced by colostrum whey. Samples of colostrum were thawed after removal from storage at -25° and 100 ml. were warmed to blood heat; 3.5 ml. of a 1:10 dilution of a commercial rennet extract were then added. After 10 min. the resulting curd was cut, and the whey was filtered off, bottled and

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stored at -25° until required. Globulin fractions G 2, 4, 5 and 6 and whey fractions WF 2, 4, 5 and 6 were prepared from the same samples of colostrum respectively. Our earlier experiments had shown that a similar degree of protection for the calf was afforded by the globulin-containing whey as by whole colostrum (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949; Aschaffenburg, Bartlett, Kon, Walker *et al.* 1949; Aschaffenburg *et al.* 1951*a*.) In the mouse-protection tests, for each strain tested the quantity of a 20 hr. broth culture that, after intraperitoneal injection, killed about half the mice injected was calculated, and was taken as the LD₅₀ dose: this was mostly about 0.12 ml. Mice were inoculated intraperitoneally with the LD₅₀ dose of test organisms 2 hr. after injection by the same route of 0.5 ml. of colostrual whey diluted 1:5–1:20 according to its titre. The mice were observed for 72 hr. after inoculation, since in preliminary trials no deaths occurred later than this.

RESULTS

Agglutination, agglutinin-absorption and mouse-protection tests. Agglutination and mouse-protection tests showed that whey from colostrum may be examined in the same manner as immune serum (see Briggs, 1951). The 'K' antibody content of colostrum and its protective activity in mice were found to be closely related. Examples with three known strains of *Bact. coli* are given in Table 1. Agglutination tests against suspensions prepared in the three different ways were usually sufficient to determine the presence of a specific 'K' antigen; occasionally, as with serum tests, recourse had to be taken to agglutinin-absorption tests.

Table 1. *Results of agglutination and mouse-protection tests with four samples of colostrual whey, and strains of Bact. coli recovered from calves (see Briggs, 1951)*

Strain no.	Test	Colostrual whey no.			
		1	2	3	4
5(a)	'K' ('L') agglutination	—	—	640	—
	'O' agglutination	—	1280	—	—
	Mouse mortality	N.T.	31/70	6/70	40/70
10	'K' ('L') agglutination	—	640	—	—
	'O' agglutination	—	—	320	—
	Mouse mortality	5/10	0/10	4/10	N.T.
25	'K' ('L') agglutination	1280	—	—	—
	'O' agglutination	—	—	40	—
	Mouse mortality	1/10	N.T.	4/10	6/10

In order to differentiate between 'K' ('L') and 'O' agglutination, the suspensions used for agglutination tests were: 1, living, 2, heated at 100° for 1 hr; 3, heated at 120° for 2 hr. The titres in the agglutination tests are expressed as the reciprocals of the dilutions. — = no agglutination at 1:10. Each mouse received one LD₅₀ dose of the test organism. The mouse-mortality figures give the mortality as the numerator and the number inoculated as the denominator. N.T. = not tested.

The table shows that colostrum containing agglutinins against the 'K' antigen of a strain protects a high proportion of mice against the corresponding strain of *Bact. coli*, that colostrum containing agglutinins against the 'O' antigen affords little protection, and that a sample containing agglutinins against neither type of antigen has no protective activity. It may be concluded, therefore, that the protective power

of a sample of colostrum against a given strain of *Bact. coli* can be estimated by ascertaining its titre of 'K' antibody.

Bact. coli as a cause of white scours. There has been some doubt concerning the role of *Bact. coli* in white scours of calves; evidence of its importance additional to that already presented was obtained by the isolation of pure cultures of *Bact. coli* from the jugular blood of two sick calves, one of which died 36 and the other 48 hr. later. By the same technique, no organisms were recovered from the blood of four normal calves or from one suffering from a transient illness. These calves had been used in experiments mentioned on p. 193 of the paper by Aschaffenburg, Bartlett, Kon, Terry *et al.* (1949).

The mechanism of the protective action of colostrum. An epidemic of white scours occurred during experiments in 1949 designed to assess the significance of lecithin in the diet of the newborn calf. Calves given colostrum and expected to live died, as well as those deprived of colostrum. A total of thirteen calves, eleven of which had received 400 ml. colostrum in their first feed, died within a period of 15 days. The opportunity was taken to examine the strains of *Bact. coli* isolated from these calves and to test the relevant 'K' antibody content of the samples of colostrum used. Examination of the strains of *Bact. coli* recovered from the heart blood and bone marrow at post-mortem showed that possibly no more than three or four serological types were responsible for the epidemic. None of the four samples of colostrum which had been bulked for feeding to the calves contained agglutinins against the relevant 'K' antigens, except possibly in two cases (6c and 9c) in Table 2, but in 6c, the calf had been deprived of colostrum. The details are given in Table 2.

The spread of *Bact. coli* from calf to calf has been demonstrated and it is reasonable to assume, therefore, that surviving calves are exposed to infection with strains that kill calves in the same environment. Evidence of a relationship between the protection of calves by colostrum and the presence in the same colostrum of agglutinins against the 'K' antigens of these particular strains, would support the view that the protective action of colostrum is of a specific immunological character.

The results of calf experiments designed to test the protective value of small quantities of colostrum proteins have been reported by Aschaffenburg *et al.* (1951a): all calves deprived of colostrum died; a few that had received colostrum or one of its fractions also died (see p. 174 of the paper by Aschaffenburg *et al.* (1951a)). An attempt was made in the present study to determine whether the presence of 'K' antibody in the colostrum or its fraction used for feeding was correlated with the survival of the majority of the experimental calves.

Accommodation for experimental calves was limited, and relatively few could be subjected to the different treatments at the same time. Since the types of *Bact. coli* vary in experiments in which fresh calves are continuously being introduced, the fractions of colostrum used for feeding were tested against the strains of *Bact. coli* isolated from dead calves that had lived in and been subjected to the same environment at the same time as those that survived. The relevant data are given in Table 3.

The data in Table 3 show that the respective agglutinin titres of globulin fractions G2, 4, 5 and 6 and whey fractions WF2, 4, 5 and 6 were similar. In general, the

Table 2. The 'K' and 'O' antigens of strains of *Bact. coli* from fatal cases of disease in calves, and the corresponding antibody content of the samples of colostrum fed

No. of calf and of strain	Colostrum	Bacterial suspensions	Agglutination tests										Interpretation of results		
			Immune serums					Colostrum whey no.							
			RR	H	JJ	TT	LLL	1	2	3	4	Antigens of strain	Antibody content of colostrum		
3c	+	1 3	—	—	—	160	—	—	—	—	—	—	—	'K' and 'O'	None observed
4c	+	1 3	—	—	—	—	—	—	—	1280	—	—	—	None observed	'O' alone
5c	+	1 3	—	—	—	—	—	—	—	—	—	—	—	'O'	None observed
6c	o	1 3	—	—	—	—	320	—	—	—	—	—	—	Probably 'K' and 'O'	Probably 'K' and 'O'
7c	+	1 3	—	—	—	—	320	—	—	—	—	—	—	'K' and 'O'	None observed
8c	+	1 3	—	—	—	—	—	—	—	2560	—	—	—	None observed	'O' alone
9c	+	1 3	—	—	—	—	320	160	640	—	—	80	—	Probably 'K' and 'O'	Probably 'K' and 'O'
10c	+	1 3	—	—	—	320	—	—	—	—	—	—	—	'K' and 'O'	None observed
11c	+	1 3	—	—	—	—	—	—	—	—	—	—	—	'O'	None observed
12c	o	1 3	—	—	—	—	—	—	—	—	—	—	—	'O'	None observed
13c	+	1 3	—	—	—	—	—	—	—	—	—	—	—	None observed	None observed
14c	+	1 3	—	—	—	—	—	—	—	—	—	—	—	'K' and 'O'	None observed
15c	+	1 3	—	—	—	—	—	—	—	1280	—	—	—	None observed	'O' alone

The suspensions used for agglutination tests were: 1, living; and 3, heated at 120° for 2 hr. Suspensions heated at 100° for 1 hr. were not used and the type of 'K' antigen ('L' or 'A') was therefore not determined. Serums were diluted from 1:10 to 1:320 and colostrum from 1:10 to 1:2560; the titres in the agglutination tests are expressed as the reciprocals of the dilutions: — = no agglutination at 1 in 10. + = calf was fed colostrum samples 1, 2, 3 and 4 bulked. o = calf did not receive colostrum.

fraction of colostrum given to a surviving calf possessed agglutinins against the 'K' antigens of the strains of *Bact. coli* against which it was tested. The titres were relatively low, possibly because the cows providing the colostrum were not the dams of the calves used nor were they necessarily from the same herd.

Table 3. *The agglutination of strains of Bact. coli from dead calves by fractions of colostrum given to surviving calves exposed at the same time to similar environmental conditions*

No. of calf and of strain	Disease and age of calf at death	Colostrum	Bacterial suspensions	Agglutination tests Fractions of colostrum			Interpretation of results
				Globulin: 5 and 6	Whey: WF 2, 4, 5 and 6	Whey: Col PP 4, 5 and 6	
23d	Typical, 2 days	o	1	40	80	N.T.	'K' ('L') agglutinins
			2	—	—	N.T.	
			3	—	—	N.T.	
24d	Atypical, 4 days	o	1	20	40	N.T.	'K' ('L') agglutinins
			2	—	—	N.T.	
			3	—	—	N.T.	
26d	Atypical, 7 days	+:G	1	40	80	N.T.	'K' ('L') agglutinins
			2	—	—	N.T.	
			3	—	—	N.T.	
29d	Atypical, 5 days	+:WF	1	10	80	N.T.	'K' ('A') agglutinins
			2	20	80	N.T.	
			3	—	—	N.T.	
30d	Typical, 2 days	o	1	80	160	N.T.	'K' ('A') agglutinins
			2	80	80	N.T.	
			3	—	—	N.T.	
31d	Atypical, 5 days	+:G	1	80	320	N.T.	'K' ('A') agglutinins
			2	80	160	N.T.	
			3	—	—	N.T.	
32d	Typical, 6 days	o	1	160	320	N.T.	'K' ('A') agglutinins
			2	160	320	N.T.	
			3	—	—	N.T.	
34d	Typical, 21 days	o	1	—	—	160	'K' ('L') agglutinins
			2	—	—	—	
			3	—	—	—	
36d	Atypical, 9 days	+:G	1	160	160	320	'K' ('A') agglutinins
			2	80	160	320	
			3	—	—	—	
38d	Typical, 9 days	o	1	—	—	80	'K' ('L') agglutinins
			2	—	—	—	
			3	—	—	—	
39d	Typical, 12 days	o	1	—	—	160	'K' ('L') agglutinins
			2	—	—	—	
			3	—	—	—	
40d	Typical, 14 days	+:WF	1	320	160	320	'K' ('L') agglutinins
			2	—	—	—	
			3	—	—	—	

The disease from which a calf died is referred to as: Typical=typical white scours with *Bact. coli* septicaemia. Atypical=colibacillosis with peritonitis and pleurisy. o=calf did not receive colostrum: control. +:G=calf received colostral fraction—globulins. +:WF=calf received colostral fraction—whey WF 2, 4, 5, 6. The suspensions used for agglutination tests were: 1, living; 2, heated at 100° for 1 hr.; 3, heated at 120° for 2 hr. The titres in the agglutination tests are expressed as the reciprocals of the dilutions. —=no agglutination at 1:10. N.T.=not tested.

Of the twelve strains of *Bact. coli* used in the tests, seven were from calves that had not received colostrum or one of its fractions; five were from calves that had received such a fraction. Six of the seven calves that had been deprived of colostrum died from typical white scours with a *Bact. coli* septicaemia; four of the five calves receiving a colostrum fraction died from a colibacillosis in which there was a marked fibrinous reaction on the pleura and peritoneum. This latter type of colibacillosis might indicate some increased resistance on the part of the individual calf. The evidence is not conclusive, but there appears to be some relationship between the agglutinins against 'K' antigens in the fraction of colostrum and a possible increase in resistance in calves that received it.

DISCUSSION

The value of colostrum to the newborn calf and the possible mechanism of its protective action have been considered in earlier papers (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949; Aschaffenburg, Bartlett, Kon, Walker *et al.* 1949; Aschaffenburg *et al.* 1951*a*). Briggs (1951) showed that most of the strains of *Bact. coli* isolated from calves with white scours possess 'K' antigens, and that these antigens are linked with pathogenicity. Whatever other causes may be involved in this disease, *Bact. coli* is usually the killing factor.

Since 'K' antibody in colostrum appears to be the factor that protects mice against experimental infection, it should be possible to determine the efficacy of a sample of colostrum against a particular strain of *Bact. coli* by agglutination tests. It was shown by this method that colostrum given to calves that died in an epidemic of white scours was deficient in agglutinins against the 'K' antigens of the strains responsible for the deaths of the calves.

In a further experiment, most of the calves receiving colostrum that contained agglutinins against relevant 'K' antigens survived exposure to *Bact. coli* which killed control calves not given colostrum in the same environment. In these experiments the evidence is not complete, but it is suggestive of the immunological nature of the protective action of colostrum. Of the twelve calves supplying the strains of organism, five received a fraction of colostrum containing the relevant agglutinins; these calves were expected to live. Four of them died from an unusual type of colibacillosis, in which there was a marked pleurisy and peritonitis; it is suggested that these showed a resistance greater than that of the control calves.

The evidence is strongly in favour of the immunological, and therefore specific, nature of the protective action of colostrum. Our earlier observation that a very small quantity of colostrum protein has a marked protective effect for calves (Aschaffenburg *et al.* 1951*a*) also supports this view. Work on the immunological aspects of the problem is continuing. It would be unwise to assume, on the evidence so far available, that agglutinins against the 'K' antigens of the strains of *Bact. coli* concerned represent the only factor of importance in the protection of calves by colostrum.

SUMMARY

1. Whey samples prepared from colostrum were examined for agglutinins against the 'K' and 'O' antigens of *Bact. coli*, and those samples containing agglutinins against the 'K' antigens protected mice against the corresponding strains of *Bact. coli*.
2. Samples of colostrum given to calves that died in an epidemic of colibacillosis did not contain agglutinins against the 'K' antigens of the strains of *Bact. coli* isolated from the dead calves.
3. In another experiment, protein fractions of colostrum given to fifteen calves that survived contained, in most cases, demonstrable agglutinins against the 'K' antigens of strains of *Bact. coli* recovered from contemporary calves that had died in the same environment.
4. The evidence at the moment is incomplete, but it supports the contention that the protective mechanism of colostrum is largely an immunological one and that it is associated, in colibacillosis, with the possession of antibodies to the 'K' antigens of the infecting strains of *Bact. coli*.

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PROCEEDINGS OF THE NUTRITION SOCIETY

SIXTY-SIXTH SCIENTIFIC MEETING
LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
3 MARCH 1951

NUTRITION AND THE PURE FOOD LAWS

Chairman: DR H. E. MAGEE, *Ministry of Health, Whitehall, London, S.W. 1*

Historical Aspects of the Pure Food Laws

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A century ago, in 1851, there was almost no administrative control over the purity of food. There were in existence a few statutes relating to beer, bread, tea and coffee, but no general act of any kind. In the social, commercial and administrative conditions of the time, food adulteration could hardly fail to flourish and to extend. It seems to have been a profitable business upon which anyone might venture almost without risk. We owe it to the enterprise of Mr Wakley, then Editor of the *Lancet*, that the year 1851 marked the inception of organized food control in the form of an Analytical Commission under the direction of Dr Hassall.

Hassall became well known as the first food chemist to make a systematic use of the microscope to detect fraudulent additions to food. His first triumph was the detection of chicory in coffee. It had been stated as late as 1851 by Sir Charles Wood, Chancellor of the Exchequer, in the House of Commons, that, in the opinion of three of the most distinguished chemists of the day, 'neither by chemistry nor by any other means could the admixture of chicory with coffee be detected'. In these circumstances a Treasury Minute had allowed the unrestricted mixture of chicory with coffee without declaration, the more readily since import duty was paid on both. After Hassall's work with the microscope a further Treasury Minute of 1853 prescribed the labelling of coffee and chicory mixtures in letters at least $\frac{1}{8}$ in. in height.

During the 6 years from 1850 to 1856 Hassall examined over 3000 samples of the more important foods, as well as many drugs, and found that at least 65 % of them were adulterated. In the list of adulterations which he gives one can discern a fairly sharp division into three classes. The first includes fraudulent but harmless substitutions and additions, such as chicory, roasted vegetable materials of different kinds and baked horse liver in coffee; flour in mustard, pepper and ginger; excessive water in any food that would hold it, and particularly in milk and all kinds of drinks; and cheap or inferior fruits or fruit juices in jams and preserves. The second class includes distinctly indigestible ingredients such as sawdust, husks of various kinds, iron oxide, alum, calcium sulphate, brick dust, bone ash, and other substances not usually regarded as actively toxic. The third, the most objectionable of all to the consumer, includes highly toxic mineral pigments such as compounds of lead, arsenic and

mercury; sulphuric acid, and even nitric acid, in vinegar; and poisonous bitters such as *Cocculus indicus*. It is difficult to form any conclusion as to the actual amounts of toxic substances liable to have been ingested in food at that time, since few quantitative figures are given, but as much as 0.6 % of sulphuric acid in vinegar is recorded.

The only bright spot in this catalogue of adulteration is the fact that chemical preservatives, boric acid, formalin and salicylic acid, were not used at that time, and did not appear until about 30 years later.

One very effective and courageous device adopted by Mr Wakley for ensuring that the work of his Commission would produce some result was the publication in the *Lancet* of the names of firms whose goods had been found to be adulterated. As the *Quarterly Review* for March 1855 (Anonymous, 1855) remarked, 'a gun suddenly fired into a rookery could not cause a greater commotion than did the publication of the names of dishonest tradesmen'. Altogether, over a period of several years, the names of between 2000 and 3000 traders were thus made known. In only one case were legal proceedings taken, and they were soon abandoned. As a result of these revelations the adulteration rate dropped in a few years from 65 to 25 %. In 1855 Hassall published his book *Food and its Adulterations* (Hassall, 1855) including the Reports of the *Lancet* Analytical Sanitary Commission, and the same year saw the appointment of a Parliamentary Committee, under the Chairmanship of Mr Scholefield, to investigate the whole question of food adulteration.

The Committee in their report (Great Britain. Parliament, 1856) said that they could not avoid the conclusion that adulteration was widespread. 'Not only', they said, 'is the public health thus exposed to danger, and pecuniary fraud committed on the whole community, but the public morality is tainted, and the high commercial character of the country seriously lowered, both at home and in the eyes of foreign countries.'

The next event was the passing of the Adulteration of Food and Drugs Act in 1860. This Act permitted the appointment of Public Analysts but did not make it compulsory. The first Public Analyst to be appointed was Dr Letheby, Analyst to the City of London, but in his first quarter he received only four samples, all adulterated. The Act was not successful, and became almost a dead letter.

A second Act of 1872 was more effective, in fact too much so for the food trade, who, according to Hassall, 'grew very wroth, and banded themselves together to get the Act repealed'. The third Act of 1875 was alleged by some to have been unduly influenced by commercial interests. It was noted that the obnoxious word 'adulteration' did not appear in it. The main provisions were that nothing injurious might be added to food, and that when sold the food must be of the nature, substance, and quality demanded by the purchaser. Both of these provisions were obviously of a somewhat contentious nature, and were criticized as likely to be a fruitful cause of future litigation. However this may be, the Act, with amendments and extensions and eventual consolidation, virtually remained in force for over 60 years.

At the time of the 1875 Act, adulteration of food, as measured by the number of samples reported against, was in the region of 20 %, or even higher by present-day standards. By 1930 it had fallen to 5 %. For this achievement credit must be given to

the original group of Public Analysts who were the real foundation upon which the Act rested. They had many difficulties to contend with, in a branch of science almost unexplored. Butter and milk analyses were in the early stages of development. The market was being flooded with fraudulent butter substitutes from Holland and Germany; compulsory labelling of margarine was not introduced until the Margarine Act of 1887. The presumptive limits of 3 % fat and 8.5 % non-fatty solids for milk were not officially prescribed until 1901.

Outstanding occurrences at the beginning of the present century were the outbreak of arsenical poisoning from beer in Manchester with 6000 cases and seventy deaths, and the oyster disaster at the Winchester Mayoral Banquet in 1902, when nearly half the guests contracted gastro-enteritis and four died. Shell-fish at that time were a very real danger. Out of 4000 cases of enteric fever annually in London, it was estimated that perhaps 30 % were due to shell-fish, mainly mussels. The treatment of mussels in purification tanks, coupled with the powers given to the Ministry of Health and to Local Authorities to close suspicious layings, and also general improvements in sanitation, have reduced the danger from this cause to small dimensions.

In 1906 the Foods Department of the Local Government Board was constituted, partly owing to reports of the extremely unsatisfactory conditions in which meat was packed in Chicago for export to this country. The next year, 1907, saw the passing of an important Act, the Public Health (Regulations as to Food) Act, under which the Foreign Meat and Unsound Food Regulations were made, controlling the importation of food from abroad. One difficulty encountered at that time was the diversion to human food of inedible lard or grease, sent from the United States to the Continent and there refined for export to this country as Pure Continental Lard. It was not until 1924 that it was eventually possible to extend the system of official certification to lard and other rendered fats, and thus to check this practice.

In 1890 and the following years increasing interest was taken in bacterial food poisoning, which was recognized as being due to specific organisms, of animal origin, that had found suitable conditions for survival and growth in prepared food. Many years later, about 1932, a further potent cause of food poisoning was found in the enterotoxin produced by *Staphylococcus aureus*, largely from human sources. In 1921, investigations into food poisoning, which had hitherto been rather disjointed, were organized into a definite scheme of research with the help of the Medical Research Council, a scheme which was eventually taken over and further developed by the Emergency Public Health Laboratory Service.

The most serious of all forms of food poisoning—botulism—has fortunately been met with on only two occasions in this country, in 1922 at Loch Maree and in 1935 in North London. Food poisoning was made compulsorily notifiable by the Food and Drugs Act of 1938.

About the year 1912 the question of a pure milk supply began to receive increased attention. The Milk and Dairies (Consolidation) Act of 1915 could not be brought into operation during the war, but in 1918 the Ministry of Food operated a tentative system of milk grading. In 1922 the first really serious attempt was made to secure the production of clean and safe milk. In that year we had the Milk and Dairies Amend-

ment Act and the Milk (Special Designations) Order with its different grades of raw milk.

Much was hoped from the grading system, which was designed to secure the full nutritive value of fresh milk without the attendant risk of infection with milk-borne diseases. It could be looked upon as the first line of defence against bacterial infection, with pasteurization as the second line. It may be questioned whether these hopes have been justified. A recent estimate gives 2000 new cases of bovine tuberculous infection annually in children with about 600 deaths and much severe crippling. Moreover, there is the unknown, but probably high, incidence of undulant fever, from both high-grade and low-grade milk. Most people seem to have come to the conclusion that compulsory pasteurization, in spite of the difficulties of enforcing it, will in the end be the only possible solution.

The history of food standards in this country dates back to 1896, when a Select Committee of the House of Commons recommended the formation of a Court of Reference to deal with definitions, standards and limits for foods. The Sale of Food and Drugs Act gave no powers to any Government department to prescribe standards in general. The powers of the Local Government Board in respect of food were derived from various Public Health Acts, and were strictly limited to the protection of the consumer's health. Standards of composition for foods in general were matters of fair trading, not of health, and a new Act would be required before the Board could attempt to lay down standards of this kind. In 1913 a bill was introduced to give the necessary powers to the Local Government Board, but it was subsequently withdrawn.

In 1919 the Local Government Board became the Ministry of Health. In 1923 standards were made for condensed milk and dried milk, but emphasis was placed upon the use of these for infant feeding and the Regulations were therefore introduced as a public health measure. In the same year the Departmental Committee on Preservatives and Colouring Matters was appointed, whose report was followed by the Public Health (Preservatives, etc., in Food) Regulations of 1925. These Regulations did an immense amount of good in clearing up dubious practices in the food trades, and are still highly effective, but they are in many respects out of date and are overdue for revision, particularly as to the meaning of the word 'preservative'.

In 1931, after representations from various bodies, there was appointed a Departmental Committee on the Composition and Description of Food, and the whole question of definitions, standards, advertisements and labelling was re-examined. The Committee was in favour of a limited number of standards, but considered that the main thing was that the public should know what they were getting.

The Committee's report in 1934 led in due course to the Food and Drugs Act of 1938, which gave much wider powers to the Ministry of Health. The Act, however, did not come into force until after the outbreak of war, and it was eventually decided that, as the Ministry of Food was in existence, all orders and regulations including food standards should be made by that Ministry.

The wartime orders made by the Ministry of Food developed into the comprehensive system of control over the advertising, labelling and composition of foods which is in

operation to-day. The court of reference so long demanded has now materialized in the shape of the Food Standards Committee of the Ministry of Food.

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Recent Advances in Food Legislation for the Protection of the Consumer

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There is a growing consciousness the world over that the consumer needs protection to ensure that the food he buys is honestly portrayed so far as descriptions and claims made in advertisements and labels are concerned and that such food should not be adulterated. These are fundamental requirements, and provide the key to the food legislation of all enlightened countries. In the United States and Canada there are only two basic food offences, misbranding and adulteration, and the regulatory definitions of these are such that almost all offences against the food laws of these countries can be taken under the one or the other of these charges.

In this country the position is not so clear-cut, but I think it can be shown that the advances that have been made since 1939 have taken us a long way along the road to our ultimate goal—the adequate protection of the consumer. The outbreak of the war prevented the Ministry of Health from exercising the potential power conferred by Section 8 of the new Food and Drugs Act, 1938. This power, somewhat reminiscent of the mantle of Elijah, was assumed by the Ministry of Food in 1943 in the form of Defence (Sale of Food) Regulations, but with several important differences.

The first is the definition of 'food'. Fitful amendments, not altogether successful, to the original definition have been made in successive Food and Drugs Acts since 1875, but none succeeded in defining the position of the borderline products—hybrids that are partly food, partly medicine, and which could claim, according to circumstance, to be either. To some degree, the position has now been clarified by the addition of the proviso that an article shall not be deemed not to be a food merely because it is also capable of use as a medicine. Borderline preparations may be thus, with sound legal authority, classified as foods.

The second is the expansion of those time-honoured words 'nature, substance or quality', three facets of the indivisible whole featured in Food and Drugs Acts for three-quarters of a century, until they were separated in the 1938 Act. Even this separation, which enables charges under one or more of these heads to be preferred under Section 3, is of no avail if false claims are made as to the nutritional or dietary value of a food, since it is by no means certain that a Court would hold that such claims were false as to the nature, or the substance, or the quality, of the food. Accordingly,

Regulation 1 of the Defence (Sale of Food) Regulations speaks of false or misleading claims as to not only the nature, substance or quality, but also, and in particular, as to the nutritional or dietary value of a food. With this extension, misleading claims in respect of almost any attribute of a food, on labels or in advertisements, may now be challenged.

The third, the solution of the difficult case of a misleading title or claim, followed elsewhere on the label by a true statement of composition of the food, was attempted. On the basis of first impressions, it was considered that no subsequent addition eliminated the misleading impression created by the primary statement, and Regulation 1 (3), supporting and reinforcing the two previous sections, contains a novel and important principle, since it states that the fact that the label or advertisement includes an accurate statement of the composition of the food shall not preclude the Court from holding that the label or advertisement is misleading. The Regulations thus provide a powerful weapon in defence of the interests of the consumer, the more so since, during the war and subsequently, important propaganda has been based on the nutritional and dietary values of foods. This type of advertising has now become a national feature and it is as true to-day as when the 1934 Committee on the Composition of Food reported, that advertising is the real power in marketing. Although the law gives power to make regulations dealing with the labelling of food, no corresponding power exists as regards advertisements, with one exception—that relative to claims concerning the presence of vitamins and minerals, where precisely the same treatment is accorded to both labels and advertisements. Until this control is extended to all types of food advertisement, the control of labels will remain but a partial remedy.

Labelling of foods

The criticism frequently levelled at the foregoing legislation is of its essentially negative character, but the exercise of the power to make food regulations constitutes a positive approach, and the Ministry of Food, since 1943, has gone quietly ahead with the task of making positive legal provisions for the protection of the purchaser. Many will be familiar with the Labelling of Food Order, which lays down the information which must now, for the first time, be disclosed to the purchaser on the labels of prepacked foods. This information includes the packer's name and address, the common or usual name (if any) of the food, and a declaration of the ingredients from which the food is made. A statement of the minimum contents of the package is required by a recent Weights and Measures Order. Perhaps the most important provision of the Labelling Order, however, is that relating to vitamin and mineral claims, whereby any such claims, whether they appear on labels or in advertisements, have to be substantiated by a declaration on the label of the identity and quantity of the vitamin or mineral present. The manner in which this declaration has to be made is laid down in detail. Rules for the labelling of alcoholic liquors are also laid down, and, as a result, a purchaser is no longer in doubt as to the origin and, to some extent, to the quality of prepacked alcoholic beverages. The old chaotic position, under which inferior products had masqueraded as high-quality articles, no longer obtains. In consequence, the purchaser is now in a position to ascertain what he is purchasing by

reading the label, and this happy position has been secured by the good work of the advisory service set up by the Ministry. This innovation is widely used, and to date well over 60,000 labels have been 'vetted'. As a result, by friendly and persuasive methods, the vast majority of food labels have been amended so that they are reasonably free from adverse comment—a result that could never have been achieved by the older methods of prosecution first and reform afterwards.

Food standards

Another major development in food legislation is that relating to food standards, but this is a movement not confined to this country by any means. Canada first entered the field of standards for food as long ago as 1890, the Food Adulteration Act, 1890, giving the Governor in Council authority to establish standards of quality for any food or drug. Increasing use of this power has convinced the Canadian authorities of the value of such standards, recently summarized so ably by Mr R. E. Curran, K.C., the legal adviser to the Department of National Health and Welfare at Ottawa (Curran, 1951): 'To-day, the purpose of a food standard is manifold. It is an assurance to the consumer that the food which he purchases is wholesome and nutritious. It is an assurance that it will not contain harmful substances nor substances which are substantially valueless to its composition. It is an assurance to the public that consideration has been given to the essential elements which a food should contain and it guarantees the presence of these elements. It is a specification to the manufacturer as to the factors which make up the worth of a food which in turn must be accepted as the criteria of its quality. These are amongst the factors which would ordinarily be considered as inherent in the purpose of a food standard.'

The attitude adopted by the United States was originally less forthright, and dependent upon the interpretation of what constitutes adulteration as set out in the Federal Food, Drug and Cosmetic Act of 1906. As a guide to the enforcement of that part of the Act, the Food and Drugs Administration issued 'standards of identity' for various descriptions of foods, but these standards, not being authorized by the Act, had no legal sanction. The insecurity of this position at law was further demonstrated by the inability of the Food and Drugs Administration to satisfy the Court that an article of food, 'Bred-Spred'—a jam-like product with a low fruit content—was adulterated, since, though it did not conform to the standard for preserves, it was sold under a distinctive name to which the Court held the standard did not apply.

In 1935, President Roosevelt, in commending new food and drugs legislation, appealed to the Senate in these words: 'In such a situation as has grown up through our rising level of living and our multiplications of goods, consumers are prevented from choosing intelligently and producers are handicapped in any attempt to maintain higher standards. Only the scientific and disinterested activity of government can protect this honor of our producers and provide the possibility of discriminating choice to our consumers.'

Accordingly, the United States Food, Drug and Cosmetic Act of 1938 gave the Administrator power to prescribe standards of composition and identity for foods, so

long as they are in the interest of the consumer, and the sanctity of standards has been invariably upheld by the United States Courts.

In this country, one or two 'standards' of sorts were included in our Food and Drugs Acts; butter, milk and cream are examples, but until the 1938 Act was passed, there was no power to make standards for foods by Regulation. The language of Section 8, in which the Minister is given power to prescribe food standards so long as they are for preventing danger to health, loss of nutritional value or otherwise for the protection of the purchaser, is strongly reminiscent of both the Canadian and United States wordings; in effect it represents a synthesis of both. In all three instances, however, the power is delegated: in Canada to the Governor, in the United States to the Food and Drugs Administrator, and here to the Minister of Food; in no case are the standards laid down in the Acts.

In the formulation of standards, the same diversity of procedure is apparent. At one extreme lies the Canadian method, where the recommendations to the Governor come solely from officials, who, however, consult trade interests on any standard beforehand. At the other extreme, the United States method is based on public meetings having the characteristics of legal hearings, at which witnesses may be cross-examined, and of which a complete record is taken. The hearing completed, the evidence is considered by the Administrator, who, before proceeding to the making of a standard, is bound to publish the 'findings of fact', as derived from the hearing. What this means is best illustrated by the assertion in 1949 of a distinguished American lawyer, that their incomplete formal hearings on the proposed standards for bread had occupied 133 days, 261 witnesses testified to the tune of some 3,000,000 words, and the transcript of evidence occupied 15,572 pages.

In this country, however, the procedure is a compromise of these extremes, we have a permanent Food Standards Committee, under the Chairmanship of the Minister's Scientific Adviser, whose members represent the Government Departments primarily concerned: the Ministry of Food, the Ministry of Health, the Department of Health for Scotland, and the Department of the Government Chemist, as well as technical and trade advisers. The Committee meets in private and examines proposals for food standards from all angles, including the fundamental one, whether a standard is necessary for the protection of the consumer. All who are likely to be affected by a standard may give evidence before the Committee. Usually, of course, this calls for the examination of trade witnesses, but in addition medical and enforcement representatives may give evidence. Ultimately the Committee reports its findings and recommendations to the Minister. If the recommendations are in favour of a standard, the report is published, so that all affected may have a further opportunity for comment. Finally, the recommendations, if accepted by the Minister, become the subject of a Food Standards Order.

As an example of yet another variant in the method of making food standards, South Africa might be mentioned. In that country, there exists a most energetic Bureau of Standards, whose energies spread well beyond the field of foods, constituted and financed something on the lines of our British Standards Institution. The Bureau, by delegating work to specialist committees, issues recommended food standards, and

these are readily adopted by food manufacturers desirous of using the Bureau's mark on the label of their products. In this respect the system has much in common with our prewar National Marks scheme.

The prescription of a standard for even a single food calls for prolonged investigatory work and the exercise of judicial qualities of no mean order. It is our experience that even under the somewhat informal British procedure, it is straining optimism to expect the production of a standard in less than 12 months. Under the more formal procedure of the United States the time lag would be even greater, but for the fact that their standards hearings proceed more or less without interruption, a procedure not possible here with a Standing Committee. Standard making is not easy, and it is not speedy, but a standard affords effective protection to the consumer. The cost (borne by Local Authorities) of legal proceedings under Section 3 of our Food and Drugs Act coupled with the reluctance of magistrates to accept as 'standards' the views expressed by witnesses for the prosecution, deter Local Authorities from taking proceedings except in those cases where fact, e.g. conformity with a legal standard, and not opinion, is at issue. Thus, quite recently one Authority declined to risk the institution of proceedings under the Food and Drugs Act when a published code of practice, agreed between the Ministry and the Trade, had been grossly violated. In these circumstances, the only real protection for the consumer is the prescription of a legal standard, and the long hard path must therefore be followed.

If this be accepted, what ought to be the characteristics of a standard of food composition? Should it, for example, deal with the complete composition of the food, and should its numerical details be fixed or should they provide ranges of variation? Or would it suffice if the chief or essential ingredients of a food were controlled within limits, leaving the manufacturer free to vary other constituents at will?

At the moment, British standards merely prescribe minimum standards to which some characteristic ingredient or ingredients must conform, e.g. 85 % spices in curry powder, without specifying the individual spices. This is certainly the simplest type of standard, but obviously one not always free from criticism. A more stringent control is the characteristic of American standards, which aim at covering the total composition of the food. To a certain extent variety is catered for by the standard including permissive ingredients in addition to obligatory ones, but to us this process savours more of standardization. If the manufacturer's product contains an ingredient not covered by these categories, his food may be held to contravene the standard, and the trader's only remedy is to persuade the Administration to amend the standard.

An important question is whether sales of substandard products should be permitted, if the labelling conveys this information. In this country it is no offence to manufacture a substandard food—the offence is selling, and selling under a description which suggests to the purchaser that he is getting a food for which a standard has been prescribed. If the description is remote from that of the standard food, ought the sale to be an offence? I must confess that we have no final views on this problem yet. In North America efforts are being made by the Food and Drugs Administration to establish that the sale of a substandard food is illegal under any label. To refer to the 'Bred-Spred' case I mentioned earlier, it must be acknowledged that it looks like jam,

it will be eaten like jam, and may be supplied as a substitute for jam. Would not, then, the case for the Preserves standard be undermined if a cheapened imitation, however speciously it is labelled, were on the market? At the moment, the American Supreme Court is wrestling with this proposition. Many of our own Food and Drugs Authorities have questioned similar cases, without risking the institution of proceedings to test their views.

Enforcement of food regulations

In enforcement matters there are also some differences in the three countries. The Dominion of Canada is covered by five district organizations, each under a technical official, centralized in the Department of Health at Ottawa, but one and the same law is operative throughout. In the United States, the Federal Law operates only in respect of food in interstate traffic, and for these goods sixteen districts, each with its own laboratory but maintaining close contact with Washington headquarters, operate. In this country, the enforcement of our law for the protection of the consumer is a duty laid by the Food and Drugs Act upon local Food and Drugs Authorities, which are independent and number over 200, and although they are advised by highly qualified Public Analysts, there is clearly room for all shades of opinion on any suggested offence, which militates against the adoption of any single line of action throughout the country.

Contamination of foods

Let us now return to the activities of our own Food Standards Committee, apart from the consideration of subjects for Food Standards Orders. The addition of substances, whether voluntary or involuntary, to food is increasing, and it is only necessary to mention the use of chemicals which may have harmful effects, or the addition of preservatives and colouring matters, by way of illustration. Subcommittees of the Food Standards Committee have now been formed, the first dealing with the omnipresent metallic contamination of foods. Already a report on arsenic has been published, and others will follow in due course on lead, copper, zinc and other metals. The second subcommittee is more recent and will consider, under the chairmanship of Professor Dodds, what changes are necessary in the present list of preservatives and other adulterants in food in Food Regulations to bring them abreast of modern knowledge and ideas.

Conclusion

It may fairly be said that energy and initiative are being shown in seeing that advantage is taken of the latest scientific knowledge in recommending food regulations for the protection of the purchaser. On this, my final comment will be a very general one, but in some measure it concerns each one of us. If the consumer is to be protected adequately, it is vitally necessary for all interested, both in food production and in the knowledge of the part good food should play in our economy, to work in season and out for complete honesty and integrity in the marketing of food. In my opinion, members of an important society such as this, have a momentous part to play in working towards the establishment of a firmly developed public opinion on these matters. Enlightened food and drugs law is of the greatest significance to the whole country, and the

knowledge of this fact is growing. True, we have not got to the stage in this country where food and drugs law has as high a priority in the legislature as it undoubtedly has in North America. But those of us who have worked in this field have reason to be thankful for the change of heart that has come about in the last few years, and in the increased interest of both trade and public. It was in order to further this interest that I accepted the Chairman's invitation to speak here this morning, and I only hope I have made a few more converts to a worthy cause.

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Problems in the Administration of the Laws Relating to the Food of Men and Animals

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The laws relating to the sale of food for man and for animals differ in certain important respects. With human food, the consumer is the main party to be protected, but with animal food, both the farmer and his livestock must have their interests protected. The Food and Drugs Act, 1938, together with the various Statutory Instruments, protect the human consumer mainly by the application of Section 3, which makes it an offence to sell to the prejudice of the purchaser anything that is not of the substance, nature and quality demanded. This Act differs from the Act of 1928 in empowering the appropriate Minister to make suitable standards which are from time to time embodied in Statutory Instruments. This newer legislation is dealt with by Adams (1951) and will not be amplified here. To a smaller extent the Merchandise Marks Act, 1926, is a protection, particularly in transactions within the food trade.

Fertilisers and Feeding Stuffs Act, 1926

The Fertilisers and Feeding Stuffs Act of 1926 differs from the above acts in being far more specific. The sale of animal feeding-stuffs must, for example, generally be accompanied by a Statutory Statement, the particulars required varying with the type of article, and being enumerated in five Schedules incorporated in the Act. In addition to the Statutory Statement, the vendor may make a voluntary statement relating to certain ingredients not scheduled in the Act, e.g. he may declare that dried grass contains over 100 mg. carotene/1000 g. This voluntary statement is binding under the Act and may also make the vendor liable to action under the Merchandise Marks Act. Regulations embodied in the Act specify limits of error that are allowed to cover small differences due to imperfect mixing and differences in analyses, for although the Regulations include methods of analysis, some variation is inevitable, due to slight differences in technique. Methods and scales for sampling are also included in these Regulations.

The two forms of legislation are similar in that they require that sampling shall be done by duly appointed inspectors, and in a particular manner. Also that the analysis

of human food shall be carried out by the Public Analyst and the analysis of animal food by the Official Agricultural Analyst. In the former instance, prosecutions are undertaken by the local Food and Drugs authority without consent of any Ministry. There are exceptions, e.g. in cases taken under Section 6, which is temporarily replaced by Regulation 1 of the Defence (Sale of Food) Regulations, or where the Enforcement Branch of the Ministry of Food institutes proceedings.

With animal feeding-stuffs, however, the permission of the Ministry of Agriculture must be given before proceedings can be undertaken. This permission is not given until the third portion of the sample has been examined and reported on to the Ministry by the Government Chemist. One of the remaining two portions is sent to the Official Agricultural Analyst for analysis and the other is given to the vendor, who may have it analysed by an independent authority if he wishes. The Government Chemist is not called upon to examine the third portion of the sample of human food unless in cases of dispute the court decides that this is necessary. The Government Chemist in both instances has legal obligations under the Acts, but whereas he acts as an independent referee reporting directly to the court on human food, his report on animal food goes to the Ministry of Agriculture before proceedings can be undertaken, and is only rarely produced in court. Proceedings under the Food and Drugs Act are normally of a criminal nature, but under the Fertilisers and Feeding Stuffs Act may be civil, criminal, or both, depending on the nature of the offence. Some of the details and workings of the latter Act may be conveniently discussed at this stage.

First Schedule of the Act

There are five schedules to the Act. The First Schedule enumerates feeding-stuffs to which all the provisions of the Act apply, and includes items such as meals made from one type of grain; cakes and meals from oilseed; copra; compound cakes and meals; meat-and-bone meal; wheat offals. Particulars that must be included in the statutory statement vary from article to article and may include oil, protein, phosphoric acid, sugar and fibre. With meals such as barley, oat, locust, pea or wheat, these details need not be supplied, but 'implied definitions' are given in the Third Schedule. As a rule the examination of First Schedule articles presents few problems since the methods of analysis are laid down in the Regulations in Part V of the Act. These methods will be revised by a subcommittee which will review the general working of the Act. It may be of interest here to state some of the analytical problems:

Moisture determination. No declaration of moisture is necessary, but in borderline cases it is essential to know that samples agree among themselves and with the bulk. The method laid down by the Regulations merely prescribes oven drying at 100°. Without going into details it is only fair to state that no single method is likely to satisfy all requirements. From many years' study of moisture determination in human and animal foods in the Government Laboratory, we have concluded that this is one of the least satisfactory of all analytical determinations. The methods include hot-air drying, freeze-drying, vacuum-oven techniques, methods which rely on distillation in presence of a solvent immiscible with water, such as toluene, under which the moisture collects and can be measured, relative humidity measurements,

desiccant methods at normal or reduced pressure and normal or elevated temperature and the Karl Fischer method.

Even the normal oven method has been modified in many ways; the latest method described by Fryd & Kiff (1951), in use for the determination of moisture in tobacco, utilizes a forced draught of preheated air. This has also been suggested by Meihuizen (1929) for dairy products and should find ready application to foodstuffs in general, where it is essential to obtain uniform results.

Protein determination. The Regulations prescribe the Kjeldahl method and the materials to be used, the catalysts being either copper or mercury. Though similar results may be obtained with either catalyst, it is our experience that mercury allows greater latitude of time of heating and gives somewhat higher results than copper. Much work has been done in recent years on the use of these, and alternative catalysts such as selenium, with pure amino-acids with the nitrogen in various forms of combination, and the results compared with those given by the Dumas method (see Alcock, 1946; Chibnall, Rees & Williams, 1943; Miller & Houghton, 1945; Reith & Wansink, 1947; Willits, Coe & Ogg, 1949). The general conclusion is that mercury gives the most reliable results and gives greater tolerance in heating.

Oil or fat determination. The Regulations prescribe extraction with petroleum spirit. This, though satisfactory for many human and animal foods, is not satisfactory for certain baked products such as biscuit meal, or with some feeding-stuffs containing molasses. We find that in such instances considerably higher results are obtained by previous hydrolysis by acid. The simple extraction method also fails to differentiate between a reasonably pure glyceride and one containing a high percentage of unsaponifiable matter such as would be expected from a meat-and-bone meal made from parts of the sperm-whale carcass. The possible harmful effect of this high unsaponifiable matter in some animals should be provided for.

Fibre determination. The method prescribed by the Regulations has been in use for many years, but slight differences in technique may influence the results in a disconcerting way. Some years ago a number of laboratories undertook a collaborative study of the method, using a sample prepared by J. F. Tocher, who afterwards carried out a statistical study. The results (Tocher, private communication) showed very clearly that the following results are inevitable where empirical methods, even though carefully specified, can lead to slight modifications in techniques. Duplicates by one worker in the same laboratory show the most consistent results. A somewhat wider spread of results is given by a number of workers in the same laboratory. Workers in different laboratories will get a much wider spread of results than either of the above.

Other schedules

The Second Schedule enumerates articles to which only some of the provisions of the Act apply, and includes clover meal, dried yeast, dried brewery and distillery grains, feeding dried blood and malt culms.

The Third Schedule enumerates ingredients, the presence of which must be declared, and includes husks, chaff, glumes, shudes whether ground or not, treated or untreated and whether used as separate ingredients or in mixtures.

The Fourth Schedule gives implied definitions for many feeding-stuffs; thus 'white fish meal' is defined as 'a product (containing not more than 6 % of oil and not more than 4 % of salt) obtained by drying and grinding or otherwise treating waste of white fish, and to which no other matter has been added'.

Harmful substances in foods

The scheduling of harmful substances both in human and animal foods is perhaps one of the most pressing of modern problems. Our own legislation differs markedly in some respects from that of some other countries, notably the U.S.A., e.g. whereas in this country the use of only five colouring matters is prohibited in human food, the American laws allow only certain scheduled colours to be used. The prevalent use of emulsifying agents, antioxidants and anti-staling agents is causing here wide concern, and although the findings of the Zuckerman Committee have not been made public, it can be stated that the legislature in both this country and the U.S.A. is now well aware of the immense biological and chemical problems confronting the Ministries of Agriculture, Health and Food.

The above Schedules were framed to enable the farmer to plan balanced rations for his livestock according to their different requirements.

Problem of determination of carbohydrate

It will have been noticed that throughout the Schedules no mention is made of carbohydrates, the farmer, unless he has had some scientific training, being left in ignorance of this factor. He cannot calculate carbohydrate from the Statutory Statement, as moisture and ash need not be declared, and fibre declared on only a few items. The calculation of 'available carbohydrate' is no less a problem in assessing the calorie value of human food, and much further work needs to be done on the human and animal organism before chemical methods can be devised that will accurately follow biological equivalents. Until recent years there was little information regarding the availability of the carbohydrates of products like ground oat husks, but the work initiated by Woodman & Evans (1938) has given us much valuable information. It should be remembered that such products are being sold as 'unrationed' feeding-stuffs to-day. Chemical analyses reported recently by Taylor (1948) showed that 'carbohydrate by difference' amounts in ground oat husks to 40-55 %. If such articles occur in a scheduled feeding-stuff their presence must be declared, as the farmer would otherwise calculate his starch equivalent quite incorrectly. We have of course precisely the same problem in compiling tables of the chemical composition of human foods. Whereas the well-known tables of McCance & Widdowson (1946) are based on the determination of 'available carbohydrate' in terms of glucose, those issued by the Nutrition Committee of the Food and Agriculture Organization of the United Nations (Chatfield, 1949) use carbohydrate calculated by difference. In many instances it does not seem possible to state which is the better, until chemical methods can be devised that will reproduce biological equivalents, and for this we need to repeat Woodman & Evans's (1938) work on man, and further to investigate direct chemical methods of

determining carbohydrates as suggested by Bransby, Daubney & King (1948). The problem reaches its most acute form in foodstuffs used by some primitive peoples, e.g. in a specimen of baobab flour which we recently examined, the carbohydrate by difference was 76 %, whereas the glucose calculated from the copper-reducing power after hydrolysis was 33 %. The exceptionally high pectin content accounted for much of the difference.

In this short review it has only been possible to refer to a few of the problems in the administration, including the framing of laws relating to foods, but it must be obvious that much remains to be done not only by the legislature but also by the Scientific Advisory Services to the Legislature before men and animals are adequately protected.

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The Toxicological Aspects of Food Adulteration

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Definition of toxicity

For the present purposes the definition of adulteration will be extended to include substances added to food for a specific purpose as well as those used as substitutes for proper nutrients. It will include also materials that find their way into food as a result of modern agricultural practices.

A substance is usually considered to be toxic if it produces some direct unfavourable effect on a normal metabolic process. In some instances the disturbance has been quite clearly defined and the nature of the 'biochemical lesion' so produced can be identified (Gavrilescu & Peters, 1931). In others injury to a vital organ results directly or indirectly from the action of a poison. Finally, a substance may be considered toxic because it is carcinogenic.

In considering the possible toxic effects of chemicals in food, their more indirect effects must not be neglected. They may act by altering the natural materials of the

food itself as exemplified by the reaction between nitrogen trichloride and wheat-flour protein. They may interfere with the function of the intestinal tract directly as, for example, by disturbing mucous secretion or indirectly by altering the dispersion of the intestinal contents and so affecting absorption. They may immobilize or prevent the absorption of important elements such as vitamins or mineral salts that are present only in small quantities.

Mode of investigating toxicity

Instead of attempting to mention the toxic properties of all synthetic chemicals used in the food industry, an outline will be given of the methods of investigation that are available, and a few examples will be chosen to illustrate the general problem.

From what has been said above, it is obvious that a simple test of acute toxicity will be of limited application for investigating the effect of chemicals used in food. It is unlikely that any material with a demonstrable, acute toxic effect when administered by mouth in reasonable doses to laboratory animals would ever be recommended for use in food. The assessment of possible carcinogenic properties is a problem quite outside the writer's experience, but on general grounds it would seem undesirable to use any material in food that has been shown to have a carcinogenic action in any species of animal.

Studies of chronic toxicity are usually made by including the test material in the diet of laboratory animals for long periods. Given the space and equipment, together with a few well-trained animal attendants, it is relatively easy to carry out such feeding procedures. The problem of assessing the results and converting the procedure into a proper experiment is quite another matter. In many instances the only index that can be used is the growth rate of the animals, their rate of reproduction and the histological appearances of the organs at the end of the feeding test. Weight changes and degree of fertility are relatively simple to determine. Evidence of histological changes in the important organs can be obtained only after careful examination of many histological preparations by a pathologist who is familiar with the variations normally found in the tissues of the particular animals being used. It is perhaps worth mentioning a few of the practical limitations to this kind of work. Considerations of space and economy exclude the use of any but small laboratory animals for such experiments. A homogeneous population must be used, and a species chosen that is not unduly susceptible to laboratory infections. The animals must be omnivorous. It can be said, therefore, that the results of long-term feeding experiments will, with few exceptions, be derived only from data obtained from rats. It would appear desirable to continue to administer the compound for as long as possible, but if the rat population is allowed to grow old structural changes in the tissues due to disease and advancing age will appear and make the task of the pathologist more difficult, if not impossible.

To determine the direct toxic effects of a compound it would be desirable to trace the history of the substance from the moment it entered the animal and to determine how much was absorbed and how much excreted and the distribution and metabolism of the compound in the tissues. For a single substance such a problem might be the work of a lifetime for a chemist and biochemist. Usually the possibility does not

exist of even attempting to carry out such a programme because there is no method for estimating small quantities of the material in the tissues. To attempt to determine the metabolic disturbances produced in the host by the presence of a foreign chemical substance would again prove a very large undertaking. If the indirect effects are considered, it might involve, for example, complete mineral-balance studies or vitamin estimations to try to determine whether or not the presence of the suspected material in the alimentary tract was interfering with the nutrition of the animal.

Whenever any information is acquired from an experiment of this type on animals, the findings have to be interpreted in terms applicable to man. The difference in the reaction of various species to drugs and chemical compounds of all kinds is only too well known to pharmacologists and physiologists. The problem of individual susceptibility is one that is particularly well recognized in man and cannot be disregarded. Before 1934 the condition known as agranulocytic angina was a clinical entity commonly fatal and of unknown origin though usually associated with a severe septic sore throat. Then an association was discovered between the incidence of the disease and previous prolonged ingestion of amidopyrine. Since that time, of course, many other drugs have been shown to be capable of producing the particular syndrome, but the drug in question has always proved to be quite harmless to the great majority of people who take it and kills only the few susceptible ones. Is it not possible that there are clinical conditions to-day that are in a position comparable with that of agranulocytic angina 20 years ago?

Examples of problems in toxicity

It is of interest to consider some examples of chemical substances found in food that are deliberately or accidentally included.

Methyl cellulose is used as a stabilizing agent, especially in ice-cream. It is devoid of any nutritive value. Rats have been given almost as much as 0.5 g., 2.5 g./kg. body-weight, daily for 9 months. Their growth rate was unaffected and the tissues were normal on histological examination (Deichmann & Witherup 1942-3). Experiments on man have confirmed the observation that methyl cellulose is not metabolized and that none of the methoxy groups are split off and excreted as methyl alcohol or formaldehyde (Machle, Heyroth & Witherup, 1944). Because it is so inert, however, it acts as an excellent laxative, and a dose of 10 g., or about 0.2 g./kg. body-weight, will double the weight of stools produced by a human subject (Tainter, 1943). Such a laxative effect might not always be desirable, but otherwise the material is probably harmless.

Polyethylene glycols of molecular weight from 1250 to 3600 have been included in foods as well as in cosmetics. Again, the evidence from experiments with rats is that large quantities, as much as 20 g./kg. body-weight daily, may be ingested for 3 months without producing ill effects (Smyth, Carpenter, Shaffer, Seaton & Fischer, 1942). Experiments with dogs have shown that they do not depolymerize the compound into the poisonous ethylene glycol but excrete it unchanged (Shaffer, Critchfield & Carpenter, 1948). The same workers have shown that the same is true for rabbits and man (Shaffer, Critchfield & Nair, 1950). In the less pure preparations of the polymers, however, ethylene glycol may be included, and it is essential that material for use in

food must conform to the highest standards of purity. Smyth, Carpenter & Weil (1950) have reported that pure preparations of polyethylene glycols have a lower toxicity than those that they tested and reported on earlier. The need to conform to the highest standards of purity will apply to all chemicals used in food, and is possibly the most important factor that should be controlled.

A number of preparations containing polyoxyethylene sorbitan monostearate have been used as anti-staling agents in bread. When incorporated into the loaf at a rate of 0.2 %, they prevent the crumbling of unwrapped bread and preserve crumb softness (Coppock, 1950). In America, where other ingredients are included in the loaf, higher proportions of the anti-staling agent must be used. It is obvious that any compound deliberately added to bread in this way must be completely non-toxic in every sense of the word.

Polyoxyethylene sorbitan monolaurate has been given to rats as from 0.5 to 2.0 % of their diet for their lifetime without apparently upsetting them, and a daily dose of 1 g. has been given to four monkeys, again without producing any obvious toxic reactions (Krantz, Carr, Bird & Cook, 1948). When, however, the monostearate was incorporated as up to 25 % of the diet of young rats, they developed nasal haemorrhages, gangrene of the tail and legs, severe diarrhoea and vesical calculi. At a level of 12.5 % the rats were unaffected (Harris, Sherman & Jetter, 1950). Though it is easy to dismiss these experiments because of the excessive quantity administered, consideration of these two reports raises the question of how to decide on a margin of safety. On the basis of the experiments just mentioned, it would seem that a tenfold reduction of a dose obviously poisonous for the rat results in an amount that can be ingested indefinitely without harm. What are the proper margins of safety to allow in calculating a safe amount for man when the only available information is the toxic and safe levels for rats? The issue with the polyoxyethylene sorbitans is further confused by a report of the effects on hamsters of ingesting polyoxyethylene monostearate at a level of only 5 % in the diet (Schweigert, McBride & Carlson, 1950). Some of the animals died, and their growth was less than that of animals given lard. In a subsequent paper the histological findings are described (Wang, McBride & Schweigert, 1950). Some of the statements made are very curious, such as that describing erosion of the mucous membrane of the intestine as being 'more intensified on one side, presumably that in contact with the intestinal contents'. Although in the text the kidney lesions are dismissed as of no significance, the photomicrograph of a section of a kidney from one of the animals shows a remarkable degree of widespread damage throughout the organ. The evidence presented may be dismissed as of no importance at all, and yet the deaths reported among the animals given polyoxyethylene monostearate may have been attributable to its presence.

Saccharin is still the most popular artificial sweetening agent, but recently others have been produced; 1-*n*-propoxy-2-amino-4-nitrobenzene has 4100 times the sweetening power of cane sugar, and reports from Holland where it was first produced claimed that it was not toxic. It is, however, a local anaesthetic, and a recent report states that when rats were fed with it, it led to changes in the thyroid gland and urinary tract and the rats developed hepatomas (Fitzhugh & Nelson, 1950). In the

same experimental conditions saccharin proved apparently harmless. Such evidence should be enough to condemn the material as unsuitable for inclusion in any food or drink.

The possible dangers from the use of carcinogenic dyes to colour food needs no stressing, and reference should be made to the work of Cook (1948) and of Kirby & Peacock (1949).

Within recent years new chemicals have been used in increasing numbers to treat growing crops and stored food products. New and poisonous insecticides are being developed in large numbers. Insecticides and pesticides are being used extensively to rid food factories and food shops of insects and other pests. Fortunately some of the most poisonous of them are also chemically unstable and rapidly disappear after being applied to food crops. Others, however, may persist for long periods of months or years, and resist the action of moderate heat. Dichlorodiphenyltrichloroethane (DDT) has been the subject of many investigations. Laug, Nelson, Fitzhugh & Kunze (1950) have claimed that rats given as little as 5 p.p.m. DDT in their diet show histological changes in their livers. The significance of the changes is, however, open to serious doubt since the fixative used was not one that would readily permit fine cytological differences to be recognized and the photomicrographs provide no support for the claims made in the paper. DDT has, however, another property shared by some, but not all, of the chlorinated aromatic hydrocarbons used as insecticides; it accumulates in the fat of animals even when the amount in the diet is as little as 1 p.p.m.

Laug, Prickett & Kunze (1950) have examined samples of human milk and fat removed from members of the general population. No appreciable amount of DDT was found in the samples of milk, but up to 34 p.p.m. was recovered from the fat of some patients. It is difficult to venture an opinion as to whether or not the continued presence in human tissues of this compound of known biological activity will be quite harmless. Tauber & Hughes (1950) found that in the rat the highest concentrations of DDT were in the lipids of the ovary, and they suggest that the observation may explain why it was the second generation of rats that was affected in breeding experiments with rats given DDT (Fitzhugh, 1948). It is extremely difficult to know what to conclude about the risks likely to accrue to mankind from ingesting food contaminated with DDT. In a period when there are food shortages in many parts of the world, hypothetical risks such as these have to be weighed against real risks of under-nourishment if pests are allowed to reduce harvests and spoil food.

More recently another group of compounds has been introduced to discourage the sprouting of potatoes kept in store for consumption at the end of the season. Tetrachloronitrobenzene is an effective sprout depressant, and it has been shown to possess little toxicity (Buttle & Dyer, 1950). Isopropylphenylurethane also has been shown to be effective for the same purpose (Rhodes, Sexton, Spencer & Templeman, 1950). It is difficult to believe that any chemical applied in small quantities to the exterior of unwashed and unpeeled potatoes before they are put into clamps for storage will ever find its way into the interior of the human body. Nevertheless, potatoes are a staple article of diet for the whole population, and their absolute freedom

from chemicals seems desirable. In considering the case of *isopropylphenylurethane*, it should be remembered that certain of the urethanes are potent carcinogens.

General considerations

From what has been said it is clear that it is no easy task to design an experimental method for determining whether a substance will be harmless if added to the diet of man. Though a certain amount of evidence can be obtained from animal experiments, the interpretation of the results must still remain a matter of personal opinion. Except for those compounds like the insecticides that find their way into food chiefly by accident, there is very little sound experimental evidence for condemning the use of any of the materials added deliberately to foods on the grounds of their toxic properties. It is unlikely that anyone will ever press for the use of a substance that can be shown by experiment on animals to possess any acute toxicity when administered in reasonable doses.

The problem of determining the effects of small quantities of a substance when ingested over a number of years, is almost insuperable unless only one particular aspect of toxicity is studied, such as carcinogenic action. When the only answer that can be gained from toxicological investigations is that of 'not proven', the decision as to whether or not a substance is to be allowed to appear in food must be based on other considerations.

On general grounds it seems desirable that the food available, particularly for urban populations, should be as nearly as possible in its natural state. Minerals and trace elements are then consumed in the same nicely balanced proportions in which they occur in living plants or animals. The production of foods which more and more resemble pure carbohydrate, pure protein or pure fat seems an undesirable trend. To add substances that may exaggerate or depress the absorption of certain ingredients may serve to upset still further the balance of the natural nutrients. One may conclude, therefore, that the problem of the adulteration of food by chemical substances will come to lie within the field of nutrition rather than in the more strictly limited one of toxicology. Nevertheless, it will still remain true that any substance added to food must display no readily demonstrable toxicity for animals and must satisfy rigorous standards of purity.

Summary

1. The meaning of the terms adulteration and toxicity is defined.
2. Consideration is given to the methods available for determining the toxicity of substances and the difficulties of deciding whether a substance can be considered harmless are emphasized.
3. A few examples of chemical substances found in food to-day are given to illustrate the different problems discussed earlier.
4. It is concluded that all chemical substances added to food must be devoid of any demonstrable acute toxicity towards mammals and must satisfy strict specifications of purity.
5. The decision as to whether or not a substance satisfying these conditions should

be used in food must rest on considerations other than those of its toxicity. Such considerations may be within the field of nutrition rather than the narrower limits of toxicology.

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Chemical Additives and Adulterants in Food

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Introductory

The question which it is proposed to discuss is one closely linked with the use of chemical additives in foodstuffs and with the potential adulteration resulting from the use of processing and hygiene aids. It is the evaluation of the functional value of chemicals, which may be suggested for these three methods of use, that will be particularly considered. Thus, only if it can be demonstrated that a chemical has a significant practical effect, i.e. a real functional value, for the purpose proposed does it become necessary to study its pharmacology in relation to the conditions of use suggested.

The discovery of an increasing number of new chemical substances has resulted in the exploration of numerous fields for their use, including the food industry, which finds itself faced with the potential use of chemicals for many purposes, some of which are outlined in Table 1. Whatever the particular use of each of these materials may be, there is the possibility that human beings may ingest them in large or small amounts, either by their deliberate inclusion as food additives, or as residues from their use at some stage in food production.

It is clear, therefore, why some of us who have become intimately acquainted with the problem have urged the necessity for precise knowledge of the pharmacology of

Table 1. *Suggested uses for various chemical substances in processes of food production*

Substance	Suggested use	Group
Methyl cellulose and other cellulose derivatives	Emulsification agent	Additive
Butylated hydroxyanisole	Anti-oxidant	Additive
'Glyceryl monostearate' containing mono-, di- and tri-esters	Crumb-softening agent in bread	Additive
Ascorbic acid	Flour improver	Additive
Propoxynitraniline (P 4000)	Sweetening agent	Additive
Alkyl and aryl silicones	Thermostable pan glaze in bread production	Process aid
Mineral oil	Lubricant for machines used in food production	Process aid
Tetrachloronitrobenzene	Agent to arrest sprouting of potatoes in clamp	Preservation of agricultural produce
DDT	Insecticide	Hygiene aid
Quaternary ammonium compounds	Detergent and bactericide	Hygiene aid
Ethyl mercuric phosphate	Fungicide	Preservation of agricultural produce

chemical food additives and adulterants, particularly their long-term chronic effects and also the effect they may have on nutrition.

In a paper read to the Society of Chemical Industry it was suggested that the pharmacologist, the chemist, the nutritionist, the clinician and the food scientist ought to have some opportunity for discussing and exploring together the problems involved in the potential introduction of new substances in food manufacture (Coppock, 1950). The health of the community may be affected by the use of chemicals as additives, and as technological and hygiene aids in food production and in the protection and preservation of crops, so that mutual exchange of information is vital. It was further suggested that the discussion of the problems which might arise ought not to be hasty, but should be conducted in an atmosphere of calm scientific approach free from all bias. For example, in America the use of certain antioxidants that reduce fat spoilage is permitted, and in considering whether their use should be permitted also in this country, it is necessary to consider their possible toxicity on the one hand and relate it to the potential carcinogenic and other effects that may be associated with the development of oxidative rancidity in heated fats; evidence for such effects has been obtained by Peacock & Beck (1948) in animal experiments with certain fats in which the process of heating can be likened to the production of accelerated rancidity.

Need for national consultative organization

The problem confronting all of us who are dealing with the science of food is one of great magnitude, and it was suggested that a national organization was necessary to co-ordinate and study its many ramifications. Guidance is required on the type and extent of the pharmacological data necessary to decide whether a substance is harmless or not. It is clear that in any system of pharmacological testing the food scientist will be required to state the potential use to which a new substance might be put,

and it will be necessary to know the concentrations likely to be used and to relate them to the scheme of testing. It appears, therefore, that many problems will require individual investigation, and broad generalizations based on the normal type of toxicological screening will rarely be sufficiently precise except as a means of preliminary sorting. Frazer (1951) has reviewed recently some of the problems inherent in the pharmacological approach which involve in many cases studies of absorption, metabolism and acute and chronic toxicity. It is probable that radioactive tracers will play an increasingly large part in the detection of contamination by new technological aids, and in the study of ingestion and of accumulation in the main viscera, more particularly where the quantities of chemicals used in foods are only small. The chemist too will be required to play his part in elaborating standards of chemical purity, in developing processes for the production of materials conforming with the required standards, and in devising adequate methods of quantitative analysis, particularly of inorganic and organic residues resulting from the use of technological aids.

It will be readily appreciated that a programme of this type, envisaging a large national organization, is not in the foreseeable future a financial possibility. Indeed, on reflexion, it appears wrong that public money should be expended in testing a large number of new substances, of which only a small number might ultimately find their way into our food. Some people may be tempted to say that because of these difficulties the use of chemicals should be completely banned. This, in the author's view, would be a wrong decision in view of the advantages that certain substances possess, and it might give rise to long discussions on such matters as the use of baking powder or the relative merits of natural and synthetic vitamins.

It is now the considered opinion of the author that two forms of initial screening can be used which would reduce to reasonable proportions the pharmacological and other work necessary to prove that the use of a chemical substance for a specifically defined purpose was free from the risk of rendering a food injurious to health. The first screening would be the simple toxicological screening referred to earlier as by itself inadequate, and it would be related to the proposed use; it would define the degree of potential acute toxicity and, depending on the nature of this finding, the degree of chronic toxicity over a defined period, say 3 months. The second form of screening would be a precise assessment of the functional value, and only if this assessment was reasonably substantiated as with, for instance, already mentioned antioxidants, would the third stage of long-term toxicity tests and studies of the effect on nutrition be undertaken. I believe that in these circumstances the pharmacological and nutritional facilities required would not be much greater than those we already possess in our universities and other research organizations. It is when this stage has been reached that some national consultative committee should consider the available evidence and express an opinion on the desirability or otherwise of permitting the use of some new chemical substance in food production and, should the committee require additional evidence, expenditure of public money to obtain it would appear well justified. The importance that proof of practical usefulness has in the suggested scheme will, however, be appreciated and, though such a scheme may involve the devising of

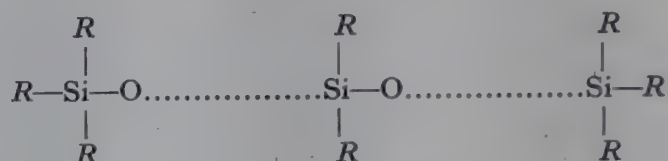
physical instruments capable of measuring selected organoleptic properties, and the development of other techniques involving the basic sciences, it is not beyond the capacity of food-research organizations successfully to carry it out.

Examples illustrating the problems that arise

Three examples will illustrate the thesis. The first deals with a processing aid in which the analytical chemist is able to demonstrate that its use for the purpose specified is without hazard. The second concerns a food additive whose functional value has been clearly demonstrated by the use of physical instruments designed to measure selected organoleptic properties. The third has been selected to show how a microbiological technique may be used in examining the efficiency of a bactericide possessing at the same time the properties of a detergent which is under examination as a potential hygiene aid.

Examination of a processing aid

The alkyl and aryl silicones which are complex molecules of the type



possess a clear-cut use in bread baking. There are still several technical difficulties that restrict their use, but they have real merit in eliminating the use of edible or mineral oils for greasing the baking tin. They are capable of producing on a baking tin a thin durable glaze of synthetic resin which can withstand repeated use for about 200 times without renewal. Previous evidence had indicated that these materials had a low degree of toxicity (Kerr, Anderson & Harris, 1949; Rowe, Spencer & Bass, 1948). In a series of comparative colorimetric determinations by the reaction between silicomolybdate and (a) stannous chloride, and (b) benzidine, it was shown that there was no significant difference between the silicon content of bread baked in silicone-treated tins and in tins greased in the conventional manner (Coppock, Hulse & Urie, 1949). The method used was a comparative one, since flour itself contains small amounts of combined silicon, *c.* 0.5 mg. Si/100 g. flour expressed on a dry basis, i.e. about 5 p.p.m. (Amos, private communication). In addition, determinations of the loss in weight from a silicone film during a series of baking experiments indicated that the maximum amount of silicone which could find its way into a loaf was 0.00005 %. It is, therefore, clear that without further toxicological information, the analytical chemist could obtain sufficient evidence that silicone pan-glaze can be used in bread production without hazard to the consumer.

The analytical data just mentioned were obtained by the British Baking Industries Research Association as part of a long-term programme of which the aim was to establish the harmlessness of various chemical additives and technological or hygiene aids that might be used in the baking industry and that might, to varying degrees, be consumed by human beings. This programme covers only a part of the whole field of

food manufacture but it has made clear to the author the need for research work of the type mentioned, and for the establishment of an official and impartial advisory committee to examine and discuss critically results of the kind that has been quoted. This aspect of the matter appears to have been considered by a recent joint committee of the Society of Public Analysts and the Food Group of the Society of Chemical Industry. In a memorandum just published, they recommended, in reference to food additives, that 'a policy should be formulated with regard to such usages, giving a list of substances permitted for these purposes' (Society of Chemical Industry and The Society of Public Analysts and other Analytical Chemists: Joint Committee on Preservative Regulations, 1951). It is most important that food manufacturers as a whole should know what substances are suitable, i.e. deemed free from health hazard, for none of them would knowingly use any chemical material as an additive or as a technological or hygiene aid if the use involved a potential danger to public health; indeed Section 1, 1a of the Food and Drugs Act (1938) defines just this position without creating any machinery to advise on the problem or assist in carrying out the provisions of the Act.

Examination of a food additive

Health considerations are involved in any form of investigation into the problem of retaining crumb softness in bread. There are many reasons for research on this problem, such as the desire of the public to buy bread that will keep fresh, the desire of the baker to produce a uniformly attractive loaf, and factors connected with production which may be associated with the changing conditions in which we live. The last set of factors is often overlooked by those who designate as sophistication the inclusion of chemical substances in food for whatever purpose, and who disregard factors that are often beyond the manufacturer's control, but which may well affect his practice. Among the substances which have been examined in America and in this country for their effect on crumb texture are lecithin, glyceryl stearates and oleates, polyglyceryl esters, polyoxyethylene stearates, sorbitan esters of fatty acids and polyoxyethylene sorbitan esters. The pharmacological properties of many of these substances have been reviewed recently in America (Lehman, 1950), and caution has been advised in the use of the three last named, not so much because of what is at present known of their pharmacology and biochemistry, as because of the gaps in information. In the proposed new American bread standards (Thurston, 1950), lecithin and the mono- and diglycerides of fat-forming fatty acids are the only ones regarded as permissible and, in each instance for reasons which would appear peculiar to America, only in association with the shortening or fat used in American bread. American bread often contains up to 6 % of fat so that the suggested inclusion of not more than 25 % glyceryl monostearate in the shortening may lead to as much as 1.5 % of it being present in the bread. This amount is considerably more than the 0.3 % which produces the optimum degree of crumb softening in British bread, for which statement the evidence will be given later.

The author, with Mr M. A. Cookson, made a particular study of the effect of glyceryl monostearate in bread of the British type. The natural presence of this

material in the bowel (Frazer, Schulman & Stewart, 1944-5), as well as the pharmacological evidence, suggest that there is little reason to expect any adverse effect on the human system. Glyceryl monostearate, as at present used, is a mixture of the mono-, di- and tristearate, the monostearate portion being usually less than 50 % of the whole. It is, therefore, closely related to natural fat and the mechanism of absorption is probably a similar one. For studying the functional value of glyceryl monostearate, instruments were constructed which could measure its effect on loaf volume, and on selected organoleptic properties that could be measured physically; such properties were firmness in relation to softness, crumb toughness in relation to chewing properties, and crumb stickiness (Cornford & Coppock, 1950).

A series of experiments was performed with a standard baking technique in which control loaves were prepared according to a basic formula, containing flour, salt, yeast and water only, and were compared with other loaves containing in addition differing amounts of a self-emulsifying glyceryl monostearate which was added as a 17 % w/v emulsion in water, the amount of water in the emulsion being deducted from the total amount used in preparing the dough. A comparison of loaves containing 0, 0.05, 0.1, 0.2, 0.3, 0.5 and 0.7 % of glyceryl monostearate expressed as percentage of the flour weight indicated that the best type of loaf was one containing about 0.3 %. The results were confirmed more precisely with the instruments mentioned and by means of other tests such as slicing experiments. Table 2 illustrates the type of results obtained and compares the effect of two preparations of glyceryl monostearate, one containing 34 % monoglyceride and 5 % sodium stearate (which confers the property of self-emulsification), and another recently marketed in America containing 90 % monoglyceride and 5 % sodium stearate. The influence on loaf volume is clear and the effect on crumb softness is equally evident on reference to Fig. 1, which compares the rate of hardening of control loaves containing no glyceryl monostearate with that of those containing the optimum concentration of 0.3 %, the materials used having the composition given above. In the same way, it is possible to determine the amounts of fat or of mixtures of fat and glyceryl monostearate which produce the optimum effect on crumb texture.

Table 2. *Comparison of the effects of two preparations of glyceryl monostearate on certain properties of bread*

Type of monostearate preparation	Volume of loaf		Toughness	Stickiness
	Control loaf with no addition (ml.)	Test loaf with added monostearate (ml.)		
Monoglyceride 34 %, sodium stearate 5 %	1358	1490	Same	{ Normal More sticky
Monoglyceride 90 %, sodium stearate 5 %	1358	1462		

In another investigation the author, with Miss R. Bennett, has shown recently that glyceryl monostearate has a pronounced effect on the improving action of vitamin C on flour. A mixture of the two substances appeared to have a synergistic action, so that

the bread made with the mixture was superior to that obtained with either material alone, even when the amount used was somewhat less than half the optimum amount for either substance alone. Similar results were also obtained with combinations of fat and ascorbic acid, as little as 10 p.p.m. of the latter being effective with a 3-4 hr. period for fermentation of the dough. The interesting speculation then arises whether the composition of the diet might not have similar effects, and suggests that there is yet much to learn about the influence of the gastric contents, in the presence and absence of food additives, on the specific absorption of dietary essentials.

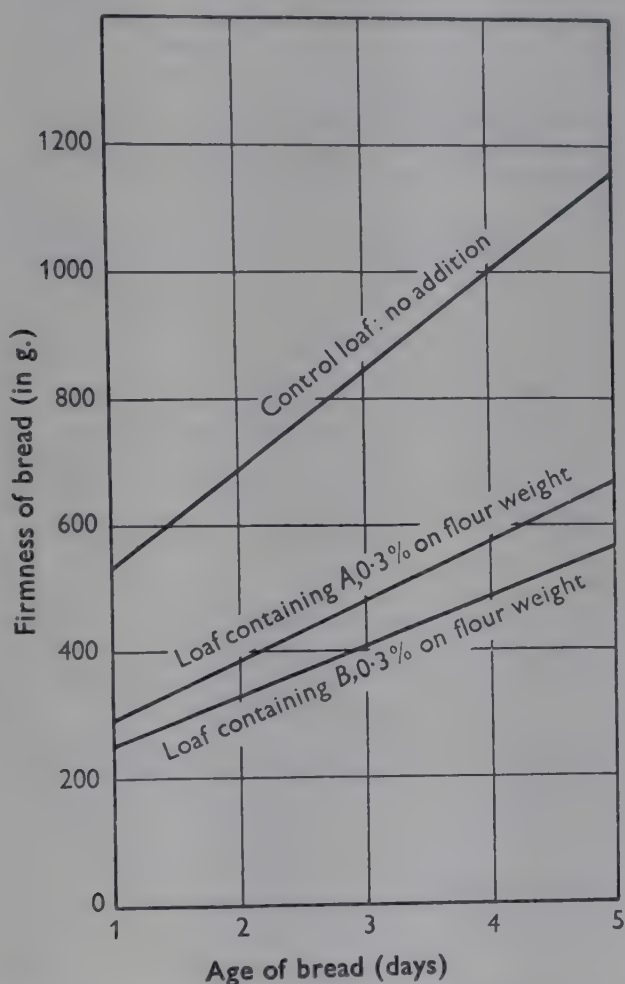


Fig. 1. Effect of two preparations of glyceryl 'monostearate', one, *A*, containing 34 % of monoglyceride and 5 % of sodium stearate, and the other, *B*, containing 90 % of monoglyceride and 5 % of sodium stearate, on the firmness of bread, expressed in g. and determined by compressing a disk of crumb 3.2 cm. in diameter and 8.02 sq.cm. in area to half its original thickness.

It will be apparent from these examples that it is possible to design experiments to provide precise information on the functional value of food additives. The pattern of this type of research is not different from that in any other form of scientific inquiry but is usually complicated by the very large number of the variables that are involved in the preparation and constitution of a food. It would of course be unprofitable to embark on a research of this scope had the initial toxicological evidence not suggested that the substance under investigation appeared to be safe for human consumption. Probably with glyceryl monostearate the wealth of information available from America makes it unnecessary to seek for further data, but the food manufacturer would feel in

a stronger position if some committee had reviewed the evidence and had agreed that the substance was a satisfactory component of food, for its use is not limited only to bread.

Application of a microbiological technique to the examination of a bactericidal and detergent substance

The third illustration has been chosen as an example of where, in the author's view, real uncertainty and doubt exist about the conditions in which it is safe to use the quaternary ammonium compounds as detergents or bactericides or as both, for which purposes they would appear to have a real functional value. Lehman (1950) regards these cationic wetting agents as possessing in general a high degree of toxicity and considers them as poisons so that their use in food cannot be justified. It is clear that their use as hygiene aids places them within the fringe of this adverse view. Fitzhugh & Nelson (1950) have demonstrated small foci of haemorrhagic necrosis in the gastric mucosa of rats ingesting 0.063 % of an alkyl-dimethyl-benzyl ammonium chloride in the diet for a period of 2 years. With Mrs D. E. Cookson, the author has recently found that cetyl-trimethyl-pyridinium bromide and the corresponding ammonium bromide lyse blood and that they are only partially removed from glass and other surfaces by rinsing (Coppock, 1950). The capacity to lyse blood is retained in acid media, and is not at all readily lost since the same effect was obtained after twelve rinses, commencing with a solution of the quaternary ammonium compound at a dilution of 1 in 10,000.

More recently a study has been made of the effect of milk and egg solids on the bactericidal properties of these compounds. In one series of experiments a suspension of *Staphylococcus pyogenes* was prepared and divided into three equal parts, to each of which cetyl-trimethyl-ammonium bromide was added, the final concentration being 1 in 5000; to one part no further addition was made, to the second was added 1 % of milk solids, and to the third 1 % of egg solids. Samples were planted at appropriate intervals on to 7 % salt agar, and counts of the bacteria present were subsequently made. The results are set out in Table 3. They show the inhibitory effect of the milk

Table 3. *Effect of a quaternary ammonium compound on Staphylococcus pyogenes in the presence of milk or egg solids. Initial concentration 200,000 organisms/g. Temperature 18°*

Time (min.)	No. of organisms in presence of cetyl-trimethyl-ammonium bromide		
	Alone	With 1 % milk solids	With 1 % egg solids
1	10,700	Uncountable	242,400
11	3,728	Uncountable	314,700
21	3,700	Uncountable	356,800
31	—	Uncountable	821,600
61	680	Uncountable	Uncountable
91	—	Uncountable	Uncountable

and egg solids on the bactericidal action of a dilute solution of cetyl-trimethyl-ammonium bromide. A similar inhibition of bactericidal properties is caused by

fats. It would appear, therefore, that in the same way the gastric contents may be capable of inactivating quaternary ammonium compounds in low concentrations and that, contrary to our earlier belief (Coppock, 1950), the potential risk of blood lysis involved in their use may be very small. It may well be, however, that, since higher concentrations of quaternary ammonium compounds are effective bactericides, there might be a temptation to increase the concentration, which might lead to the contamination of food brought into contact with surfaces treated in this way. Quantities far in excess of 1 or 2 p.p.m. might thus be introduced into food. It would be unwise, therefore, to recommend these materials for general use in the food industry until further knowledge of their properties is available, although their use at low concentrations of about 1 in 6500 for specific purposes such as rinsing beer glasses might be regarded as an exception. Results of this type further emphasize the need for a consultative committee, having this sort of information at their disposal, or having fostered facilities for obtaining it, and able, therefore, to advise generally and specifically on the safety of procedures of this type.

Conclusion

There are many further examples that could be quoted to justify the need for guidance. Sometimes it might be necessary to recommend precautions beyond those legally defined, as for instance in the advice (Coppock & Cookson, 1949) given to the baking industry about the *purity* of the mineral oil to be used for lubrication for which no specification is made in the Mineral Oil in Food Order 1949 (S.I. 614), where the *weight* of mineral oil permitted in food resulting from processing operations is given as 0.2 %. After consultation with Professor Frazer it was felt that mineral oils of high viscosity were less likely to be absorbed and possibly cause liver damage than oils of low viscosity, so that a recommendation was made that the chemical purity of mineral oil likely to come in contact with food during baking should be not less than that of liquid paraffin B.P. This example emphasizes the need for some consultative committee to examine carefully the potential uses of the substances brought to its notice, and suggests that the constitution of the committee should be wide enough to enable it to obtain the necessary evidence from the representatives of industry.

There can, in the author's opinion, be little doubt that an advisory committee of the type suggested is necessary in view of the problems revealed by this examination of some of the difficulties facing the food scientist. The problems arise not only as a consequence of the new substances that the chemical industry puts forward as likely to be useful, but also because new trends in hygiene, in modes of transport, in food preservation, in crop protection, and in packing and production yield an increasing number of instances where guidance is an urgent necessity.

The work of a consultative committee would at first be heavy because of the accumulation of problems requiring an answer, but it is the author's view that in the future many materials would be eliminated by a preliminary pharmacological screening, followed by evidence of a real practical application or functional value. The total amount of additional pharmacological and nutritional research would not then become unwieldy, and the possibility of giving helpful advice to the food producer

would be realized within rational and economic limits. Such an arrangement clearly requires the co-operation of the food producers and the food research organizations with the Ministries of Food, Health and Agriculture and, almost certainly, at some stage in the third phase of inquiry, guidance by the Medical Research Council on the need and pattern of further research.

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SIXTY-SEVENTH SCIENTIFIC MEETING—THIRTY-FIRST
SCOTTISH MEETING

HANNAH DAIRY RESEARCH INSTITUTE, KIRKHILL, AYR

21 APRIL 1951

MILK

Chairman: PROFESSOR R. C. GARRY, *Institute of Physiology, University of Glasgow*

Trends in Milk Consumption in Great Britain

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This Society last devoted a whole day to milk in July 1943 at a joint meeting with the Food Group of the Society of Chemical Industry. In the first paper at that meeting Mr J. L. Davies (1944), of the Milk Marketing Board, discussed the changes in the supplies of liquid milk which had occurred between 1933 and 1943 and posed a number of questions. Among these were: What should be the rate of liquid milk consumption to meet a progressive nutritional policy? How much milk would people buy when other foods become plentiful again? Would supplies be sufficient to meet the high nutritional requirements he postulated?

Data collected during the 8 years since that meeting suggest some answers to these questions. It is proposed here to discuss some of these data.

Trends in national supply. Before the war the Advisory Committee on Nutrition of the Ministry of Health (Ministry of Health, 1937) reviewed the production and use of milk in the United Kingdom and studied the trends which had taken place in the consumption of milk and its products during the first third of this century. The data below, which are extracted from that report, show the consumption of milk and milk products in 1909-13, 1924-8 and 1934-5:

	1909-13	1924-8	1934-5
Milk and cream (pt.)	3.46	3.35	3.26
Condensed milk (lb.)	0.05	0.14	0.23
Estimated total in equivalent pints	3.57	3.65	3.75

It will be noted that while the total consumption of milk and cream fell by some 6 % between 1909-13 and 1934-5 that of condensed milk increased between four and five times, and caused the total to increase by about 5 %. The report went on to discuss the reasons for the fall in liquid milk consumption and suggested that one might have been the change in the age distribution of the population. It also showed that between 1934 and 1936 there was a 64 % increase in the amount of milk used for manufacture, so that by 1936 as much as one-third of the total milk sold off farms was used for the manufacture of products for human consumption. In commenting on this change in the use of milk the Committee stated that 'much of the dried and condensed milk is used in confectionery and most of the remainder is in the form of condensed skimmed milk, which is not equivalent in its constituents to fresh whole milk'.

It is against this supply and utilization picture that the Advisory Committee's recommendations on desirable changes in milk consumption must be viewed. They recommended that children should receive from 1 to 2 pt. of liquid milk a day, expectant or nursing mothers about 2 pt. and other adult members of the population $\frac{1}{2}$ pt. and estimated that these allowances would have represented an average of about $\frac{7}{8}$ pt. of liquid milk daily per head of the population, an amount which was more than twice the then existing liquid milk consumption or about one and three-quarter times the total milk consumption.

The Advisory Committee did not discuss methods of inducing the population to buy and drink these quantities of milk. A start had been made many years previously in supplying free milk to needy mothers and children. In the early years of the century it could be obtained from local charities, but after the Maternity and Child Welfare Act was passed in 1918, any local authority was permitted to supply cheap or free milk to poor mothers and children against a medical certificate. In 1935-6, the Milk Marketing Board, the trade and the Commissioner for Distressed Areas co-operated in starting a cheap milk scheme for mothers and children in the Rhondda Valley, Whitehaven, Jarrow and Walker-on-Tyne, and in August 1939 the Ministry of Health introduced a national cheap milk scheme on an income basis. At the end of the twenties the National Milk Publicity Council started promoting the sales of milk in schools, and in 1934 the Milk Marketing Boards and the distributive trade, in co-operation with the Education Departments, started the Milk-in-Schools Scheme which gradually grew in importance until in 1939 over half of the pupils in grant-

aided schools were taking school milk. The children could buy one-third of a pint daily at school for $\frac{1}{2}d.$; those from very poor homes could obtain it free.

In spite of these efforts to increase milk drinking, the national consumption in 1939 was still far below the Advisory Committee's target. The outbreak of war made complete control of the national milk supplies a necessity, and therefore the initiation of a system of milk priorities a possibility. Early in the war the Ministry of Food took three main steps to implement the Government's nutritional policy on milk. It encouraged milk production; it diverted milk from manufacturing to liquid consumption; and it introduced the National Milk Scheme. The extent to which the first two objectives were achieved is illustrated below:

	1933-4	1935-6	1938-9	1941-2	1945-6	1948-9	1950-1
Total sales off farms (gal. $\times 10^6$)	950	1163	1253	1208	1405	1630	1826
Percentage for liquid market	74	65	68	89	89	89	83

Total milk sales did not begin to rise above the prewar level until 1943, but the diversion of milk from manufacture to the liquid milk supply had started to become effective by 1940, and it will be noted that after 1941 and until the present year nearly 90 % of the total milk sales have gone for liquid consumption.

The National Milk Scheme, which later became part of the Welfare Foods Service, was introduced in July 1940 and provided milk free or at a special price for all expectant and nursing mothers and young children irrespective of income. Another important measure implemented shortly afterwards by the joint action of the Ministry of Food and the Education Departments was the expansion of the Milk-in-Schools Scheme. By the beginning of 1943 about three-quarters of the school population was taking milk at school, compared with just over half in 1939. School milk was taken by about this proportion of children until after it became free of charge in August 1946. Since then nearly 90 % of school children have taken school milk.

The increase in milk production and the diversion of milk from manufacture were not sufficient to meet the requirements of the National Milk Scheme and the expanded Milk-in-Schools Scheme, and in April 1941 restrictions had to be imposed on milk purchases by adults or, as they came to be called, 'non-priority consumers'. At the same time it became necessary to arrange for special allowances of milk for certain categories of invalids, and in October 1941 a priority claim on milk supplies was also arranged for older children and adolescents. This system of allocation remained substantially unchanged until supplies of liquid milk became sufficiently plentiful in 1950 to obviate any further need for controlled distribution. When milk was fully controlled the following priority domestic allowances were available:

Priority class	Weekly allowance (pt.)
Expectant mothers	7 cheap or free and adult's allowance at full price
Children 0-1 year	7 cheap or free and 5 at full price
Children 1-5 years	7 cheap or free
Children 5-18 years	3½ at full price
Certain groups of invalids	7-14 at full price

In addition to these, all school children were entitled to $\frac{1}{3}$ pt. of milk a day at school,

and special arrangements were made for children unable to attend school or living in institutions.

These quantities were guaranteed throughout the year, but as milk supplies vary seasonally, it is clear that the amount left over for the non-priority consumers could not remain constant throughout the year: in the autumn and winter it often fell to 2 pt./head/week; in the late spring and summer it usually rose to 3 or 4 pt./week. At certain seasons of the year over half the total supply went to priority consumers (comprising about a quarter of the population), compared with just under 40 % when

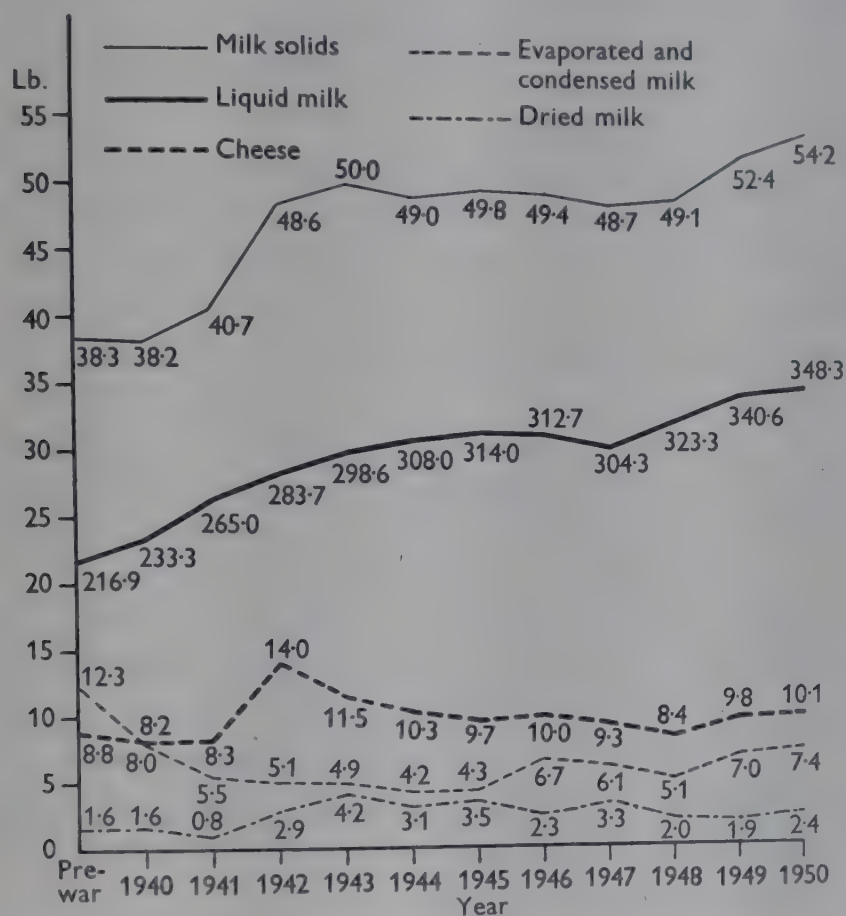


Fig. 1. Supplies of milk and milk products moving into civilian consumption in lb./head/year in the United Kingdom, prewar to 1950.

milk was most plentiful (Capstick, 1947-8). Milk was decontrolled in January 1950 and after that date the only priority allowances were the 7 pt. weekly supplied cheap or free under the Welfare Foods Service to expectant mothers and children up to the age of 5, and school milk.

The trends in the national supplies of milk and milk products that have occurred since 1939 can be seen in the estimates of the 'total supplies of foods moving into civilian consumption' which are published from time to time. The most recent appeared in a White Paper *Food Consumption Levels in United Kingdom* (Ministry of Food, 1949). The average total supplies for the United Kingdom of liquid milk, milk products and total milk solids (including cheese) have been extracted from this report and, with some as yet unpublished later estimates, have been graphed in Fig. 1; the points on the graph represent yearly averages. From this graph it will be seen that

total milk solids have increased by over a third between 1939 and 1950 and liquid milk itself by over half. The increase in liquid milk has been fairly uniform except for a slight setback in 1946, and a more serious shortage in 1947 caused largely by the very cold winter of 1946-7 followed by the prolonged drought in the summer of 1947. Total milk solids have shown a different trend with a sharp increase in 1942 caused mainly by the increased imports of cheese and dried skimmed milk after the passing of the Lend Lease Act in the United States. It will be noted that the 1950 milk-solids figure is almost up to the level (55.5 lb./head/year) recommended in 1947 by Dr Magee and his collaborators as a desirable target for this country (Bransby, Magee, Bowley & Stanton, 1947). It is also of interest to note that although the average supply of cheese was lower in 1950 than in 1942, it is still significantly higher than at the prewar level: 10.1 lb./head/year compared with 8.8.

This paper has, so far, dealt only with national supply trends and the provisions that have been made to enable mothers and children to obtain the milk that has been recommended for them. Other data on milk consumption are, however, available and will now be discussed.

Data on milk consumption. In 1940 the Ministry of Food initiated a National Food Survey which has been carried on continuously since that time. The first report on the results of this survey, which is now in the press, gives full details of techniques used, but it may be useful to state briefly that for the greater part of its history the survey has provided records of the domestic consumption of foods and expenditure on foods bought for use in the home by urban working-class households.

The results during 1940 and 1941 (Ministry of Food, 1941, 1951*a*) are particularly relevant to any study of milk consumption as they throw some light on the changes which took place after the National Milk Scheme came into force and after the supply of milk for adults was restricted. Table 1 shows some of the trends which were taking place during 1941. The figures for 1940 have not been included as they resemble closely those for the early months of 1941.

Table 1. *Average consumption of household and school milk*

(National Food Survey of 1941, Ministry of Food, 1941)

	8 Jan.-16 Feb.	28 Apr.-26 June	28 July-6 Sept.	27 Oct.-6 Nov.
Average consumption (pt./head/week):				
All households	3.3	3.2	3.4	3.2
Households without children under 14	3.6	3.3	3.6	3.1
Households with children under 14	3.2	3.2	3.2	3.3
Average consumption of National Scheme milk per child under 5:				
Pt./week	5.8	6.3	6.4	6.5
Percentage of entitlement	83	90	91	93
Average consumption of school milk per child aged 6-14 (pt./week)	0.7	1.0	0.6	1.4
Milk obtained under special schemes as percentage of total milk	18	19	18	22

One effect of the restriction on the supply of milk to adults at a time when the amount of National Scheme milk and school milk taken was increasing, was to raise the average amount consumed per head in households with children above that in childless households. This difference in favour of households with children appeared for the first time in the fourth quarter of 1941 when households with children bought an average of 3.3 pt./head/week and childless households 3.1, compared with 3.2 and 3.6 pt./head/week at the beginning of the year. By the fourth quarter of 1941, 22 % of all liquid milk supplied to surveyed households, all of which were urban working-class households, was obtained under these schemes. The average amount of National Scheme milk was 6.5 pt. a week for each child under 5, and of school milk 1.4 pt. for each child aged 6-14.

Although sales of milk off farms did not rise above the prewar level until 1943, the reduction in the quantities processed into cheese and other milk products made possible a steadily increasing liquid milk consumption by urban working-class households from 1941 onwards. The records of the consumption of milk by the urban working-class households surveyed are summarized in Table 2 which is extracted from the report of the Ministry of Food (1951*a*) already referred to.

Table 2. *Domestic consumption of liquid milk, expressed as pt./head/week*

(National Food Survey of 1942-9, Ministry of Food, 1951*a*)

Year	Milk retailed at full price	National Scheme milk	School milk	Total	Average non- priority allowance	No. of households	No. of surveyed persons
1942	2.6	0.7	0.2	3.5	2.5*	8567	29,493
1943	2.8	0.9	0.3	3.9	2.6	9141	31,733
1944	2.9	0.9	0.2	4.0	2.5	7263	26,015
1945	2.9	0.9	0.3	4.1	2.4	7225	24,968
1946	2.9	0.9	0.2	4.0	2.4	8204	29,260
1947	2.8	0.9	0.2	3.9	2.4	5942	21,334
1948	2.9	0.9	0.2	4.0	2.5†	5623	20,178
1949	3.3	0.9	0.2	4.4	2.7‡	7119	25,737

* Unrestricted sales for 17 weeks.

† Unrestricted sales for 3 weeks.

‡ Unrestricted sales for 15 weeks.

These results show that by 1945 the average consumption among the urban working-class households surveyed was 4.1 pt./head/week, or more than one-third greater than the average of 3.0 pt. recorded in 1937-8 by the Ministry of Labour (1940). This increase is, of course, to be attributed partly to the wartime rise in incomes, but it was partly the result of the Milk Schemes. In the last quarters of 1944 and 1945 National Scheme and school milk together represented 30 % of all milk consumed by the surveyed households, compared with the 22 % for the last quarter of 1941.

After the war the continuous increase in milk consumption by urban working-class households suffered a slight check owing to supply difficulties, but by 1949 it had risen to 4.4 pt./head/week or nearly 50 % more than before the war. The increase from 1948 to 1949 was largely in the form of milk retailed at full price and represented an

improvement in the diet of the general consumer. It is important that this should have happened at a time when the diet was becoming more varied, although it must be remembered that at its subsidized price milk was still economically attractive compared with many of the more expensive foods responsible for the increased variety of the diet.

Survey of May-June 1949. Until milk was decontrolled it was impossible to tell whether or not the 50 % increase in milk supplies between 1939 and 1949 was sufficient to meet current demand. Nor was it possible under restrictive conditions to gauge how people in different income groups shared in the general increase. When milk supplies were sufficient to permit temporary decontrol, between the end of March and mid July, 1949, the Ministry of Food took the opportunity to seek an answer to these questions in an *ad hoc* consumer survey. The results of this survey have already been published by the Ministry of Food (1950*a*) but they will be reviewed briefly here.

The survey covered 2400 households containing 8600 people, broadly representative of all economic groups, urban and rural areas being represented in their correct proportions. Housewives were asked how much milk they were buying and whether it was more than they had been buying before milk was decontrolled; if they were not buying more they were asked why.

The results of the survey showed that the supply was adequate to meet the demand at the then current prices: only 1 % of the housewives said they were unable to buy more because the milkman could not deliver it. This does not mean, however, that all families could obtain all they wanted, because of those housewives who had not increased their milk purchases to any marked extent after decontrol 23 % (17 % of the total sample) did not do so because of its cost. This proportion varied from 5 % in the highest income group to 41 % in the lowest.

The average domestic consumption per head per week of all consumers was found to be about 4 $\frac{3}{4}$ pt. Assuming that priority consumers were taking up their allowances in full, this meant that the weekly consumption of non-priority consumers averaged about 4 pt./head. It is of interest to compare these results with those found in surveys made before the war. A comparison is made in Table 3.

Table 3. *Domestic purchases of liquid milk, May-June 1949 and according to prewar surveys, expressed as pt./head/week*

Income group	Average purchases			
	Crawford & Broadley (1938) 1936-7	Orr (1936) 1934	Ministry of Food (1950 <i>a</i>) 1949	
			(a)	(b)
A	5.1	5.5	6.0	5.8
B	4.4	4.2	5.5	5.3
C	2.6	2.6	4.5	4.8
D	1.6	1.1	4.5	3.7
All households	2.9	3.1	4.8	4.8

Crawford & Broadley and Ministry of Food (a) groupings based on income of heads of households.

Orr and Ministry of Food (b) groupings based on average income per head.

Comparison cannot be exact since the procedures used in the prewar and postwar surveys were not identical. The only two published prewar surveys that can be used for an income-group or social class comparison are those of Orr (1936) and that of Crawford & Broadley (1938). Orr's data were based on surveys conducted from 1932 to 1935 and his national estimates made for 1934, and Crawford & Broadley's covered the months October 1936 to March 1937. Income groups in Orr's *Food, Health and Income* were based on estimated average income per head in the family, in Crawford & Broadley's *The People's Food* on the incomes of heads of households. Orr's data on milk covered total consumption, including milk drunk in catering establishments and schools, and Crawford & Broadley's covered consumption in the home only. However, despite these differences and the fact that neither of the prewar surveys was seasonally suited for comparison with milk consumption in May-June 1949, it is of interest to draw up a comparison between the three sets of figures. In Table 3, group A is the highest income group and group D the lowest. In the three surveys group A comprises 5-10 % of the sample, group B about 20 %, group C about 60 % and group D 10-15 %.

In spite of the inexactness of the comparisons the broad result is unmistakable. According to the prewar surveys the upper 5-10 % of the population drank more than three times as much milk as the lowest 10-15 % and about twice as much as the main working-class group, who represented 60 % of the population. In May-June 1949 the difference between the groups was strikingly smaller: the highest group was then consuming little more than one-third to one-half more than the lowest. To express this another way, while the whole population had on the average increased its consumption by one-half compared with before the war, working-class consumption had nearly doubled and that of the poorest had trebled.

National Food Survey. The trends in milk consumption have been studied further since the survey of May-June 1949. The National Food Survey which had previously covered mainly urban working-class households was reorganized in 1950 so that it would include all sections of the population, and from the results of this survey it is possible to compare the total milk consumption (including school milk and processed milks) by families of different social class and different size. The months during which the survey took place in 1950 were January, February, April, May, July, August, October and November. The total number of households surveyed was 4723: these contained 16,554 persons. The data which follow are taken from unpublished reports of the Ministry of Food (1951*b*).

In Table 4 the consumption of liquid and processed milk by social classes is compared with that of the similar social classes surveyed by Crawford & Broadley (1938). This table shows the same broad result for liquid milk as the previous one. In addition, it will be seen that in 1950 the class A households received a quarter more of all milks than they did in 1936-7; the class B households a third more; the class C households three-quarters more; the class D households one and a quarter more and all households two-thirds more. It was known that the lowest income groups relied considerably on sweetened condensed skim milk before the war. In 1950, however, this product accounted for less than a quarter of the working-class consumption of processed milk:

Table 4. *Household consumption of milk and milk products according to social class expressed as pt. or equivalent pt./head/week. 1950 compared with 1936-7*

Social class	Percentage of population		Average consumption					
	Crawford & Broadley (1938) 1936-7	Ministry of Food (1951b) 1950	Liquid milk		Processed milk		Total	
			1936-7	1950	1936-7	1950	1936-7	1950
A	5	3	5.1	6.3	0.05	0.2	5.2	6.5
B	20	13	4.4	5.4	0.1	0.4	4.5	5.8
C	60	58	2.6	4.7	0.3	0.4	2.9	5.1
D	15	26	1.6	4.4	0.5	0.3	2.1	4.7
All households	100	100	2.9	4.8	0.3	0.4	3.2	5.2

three-quarters was made up of about equal quantities of whole condensed or dried milks. The nutritional importance of the increased consumption in the lowest income groups is therefore even greater than appears from this table.

An analysis of the variation in milk consumption between households of different size also has been carried out on the results of the 1950 survey. The most extensive prewar survey which studied the effect of family size on milk consumption was that in 1938 and 1939 of Murray & Rutherford (1941). These workers showed, however, that although incomes and the numbers of adults and children in the household affected milk consumption, these factors were not the whole explanation of the divergences found. Regional differences and habit and taste factors were also important. It has not been found possible to compare the present data on family size with those of Murray & Rutherford and therefore no prewar comparisons can be made with the 1950 findings, which are shown in Table 5.

Table 5. *Household consumption of milk and milk products according to family composition, expressed as pt. or equivalent pt./head/week*

(National Food Survey of 1950, Ministry of Food 1950b)

No. of persons per household	Household composition	Consumption				Requirement†
		No. of households surveyed	Liquid milk including school milk	Other milk	Total	
2	1 man, 1 woman only*	688	5.5	0.3	5.8	3.6
3	1 man, 1 woman, 1 child†	634	5.3	0.5	5.8	5.0
4	1 man, 1 woman, 2 children	567	5.1	0.5	5.6	5.7
5	1 man, 1 woman, 3 children	198	4.9	0.6	5.5	6.0
6.5	1 man, 1 woman, 4 or more children	112	4.3	0.6	4.9	6.4
3.2	1 man, 1 woman and adolescents†	214	4.8	0.3	5.1	4.8
5.4	1 man, 1 woman and adolescents and children	331	4.2	0.3	4.5	5.8

* Excluding all old-age pensioner households.

† Child = under 14 years, adolescent = 14-20 years, adult = 21 years and over.

‡ Calculated according to Advisory Committee on Nutrition recommendations (Ministry of Health 1937).

It will be seen from this table that the households containing adults only drank more liquid milk per head than any of the other types of households, but that if processed milks are taken into account the households containing one child under 14 equalled the childless households and the households containing two and three children almost did so. The large families and those containing adolescents fared worse.

The Ministry of Health (1937) Advisory Committee's recommendations on the amount of milk required by people of different ages have already been mentioned. The requirements of the average families shown in Table 5 have been calculated on the assumption that children aged 0-1 should drink 2 pt. of milk a day and all other children and adolescents up to the age of 21, 1 pt., expectant mothers 2 pt. and other adults $\frac{1}{2}$ pt. It will be seen, that, according to this calculation, childless households, households containing one child and households containing adolescents were apparently meeting their milk requirement, households containing two or three children were almost doing so, households containing four or more children and those containing both children and adolescents were failing to do so.

No earlier data with which to compare these estimates are available; it is therefore impossible to suggest trends for families such as these. The numbers of large families studied during 1950 were small, but the figures for each quarter were remarkably consistent and showed that families containing two adults and three or more children or adolescents did not buy the quantity of milk that has been recommended even though milk is now readily available. It is not easy to determine whether this is due to the high milk bill in spite of the very cheap rate at which milk for children can be obtained or whether it could be remedied by education. It is important to realize, however, that this difference between large and small families is almost certainly not a new situation, and the milk consumption of large families is probably greater than it was before 1939, but the fact that they are getting less than their share of milk in spite of the Welfare Foods Service and the Milk-in-Schools Scheme suggests that more education in milk drinking is required.

SUMMARY

The trends that have been discussed may be summarized as follows:

1. As a result of increased production and the diversion of milk from the manufacturing to the liquid market, liquid milk consumption in Great Britain has increased by over 50 % between 1939 and 1950.

2. Largely as a result of the Government's distribution policy, combined with greater purchasing power among the poor, the gap between the milk consumption of the upper and lower income groups has been narrowed.

3. It appears that in addition to special incentives in regard to price, more education may be necessary if the Welfare Foods Service is to be fully effective in furnishing adequate supplies of milk to the larger families.

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Research in Dairying—A Survey

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Changes in Milk Production in Great Britain during the Past Half-century

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Organization of the industry at the beginning of the century

The producers of milk and dairy produce at the beginning of the century and up to the twenties could be grouped into a series of zones. This was necessitated by the comparatively primitive methods of handling and transporting milk, and also to a considerable extent by the consumers' prejudice against milk that had been cooled and delayed for more than a few hours in its progress from the cow to the breakfast table.

Cows were kept in many towns and large cities, so that milk could be delivered quickly, and be handed to the consumers fresh and warm from the byre. Often the only land attached to the 'town dairy' was an exercise yard, or paddock, so that no pasturage or arable crops were available, and all food had to be bought. This necessitated a steady supply of purchased hay, straw and roots, as well as more concentrated feeding-stuffs, and also a ready market at all times of the year for the manure. Thus, the system survived longest in the towns where, within easy carting distance, there was land well suited for arable cultivation, with farms ready to sell farm crops and to buy back manure.

The system also required the constant renewal of the cow population because breeding or rearing of stock was rarely attempted. Newly calved cows of a dual-purpose type were bought and milked as long as they continued to give a satisfactory yield. They were fed heavily, and by the time the yield of milk had dropped to about 1 gal./day, they were sold for slaughter as fat cows and replaced by new purchases. I need not say anything about the objections on the part of the health authorities to the keeping

of cows in the middle of densely populated town areas. From an agricultural point of view, the system was extremely wasteful because of the slaughter of many of the best dual-purpose cows of the country when they had barely reached their most productive period, and could have been kept for at least 2 or 3 years longer to breed calves of a similarly high class.

On the outskirts of the towns were farms which not only kept cows, but also had a certain area of land for both grass and arable crops. They, therefore, grew hay, straw and roots required by the herd, and thus their main purchases were concentrated foods such as the by-products of the oil-crushing, milling and brewing industries. Their land was, however, too limited in area and too valuable to be used for the rearing of stock, so that they also depended on the purchase of cows, though they might take a calf or two from some of the best and youngest cows. Their milk too was delivered as quickly as possible to the consumers, usually twice a day in summer, though possibly only once a day in winter. The necessity for milking at what now seem to be unearthly hours in the morning—3 or 4 a.m.—in order to get the morning's milk to the customer in time for breakfast partly accounted for the distaste which most farmers had for what they termed 'cow keeping'.

At a greater distance were farmers dependent on railways for the delivery of their milk into the towns. In the absence of proper cooling on the farms and of refrigerated vans for railway transport, it was impossible to avoid considerable wastage by souring during the hot summer months. Consequently many such farmers sold milk only in winter, and during the summer months made cheese. Apart from other considerations, the wholesale price of summer milk was often very unattractive.

At a still greater distance from a liquid milk market, cheese-making dominated the policy in areas well suited by soil and climate for milk and cheese production, but unsuited by the situation for the sale of liquid milk. The most important of these were: the south-west of Scotland with its Cheddar and Dunlop cheese; the Cheshire region, including considerable areas of North Wales, Staffordshire and Shropshire, as well as Cheshire itself; the Somerset area, which was the original home of the Cheddar cheese, and the north Midlands with Derby and Stilton cheese. Cheese-making under farmhouse conditions is mainly a summer occupation, and milk can be produced most cheaply on grass in summer. Therefore, nearly all the cows calved in early spring, March or April, and were thus ready to take full advantage of the flush of grass in May and June. They dried off in the autumn and were kept cheaply throughout the winter, almost entirely on hay, though some of the cheese-making farmers would sell some milk in winter. These men would thus have to have a proportion of cows calving in the autumn, and would feed their herd on a higher level than the pure cheese-makers.

In many areas unsuited for large-scale arable farming or for the production of milk on a large scale, dairying as a subsidiary industry was of considerable importance because the breeding and rearing of cattle was one of the most important enterprises, especially in marginal or upland areas. As a by-product, butter brought in an income to the farmer's wife, and a substantial proportion of the country's total requirements for butter was supplied by our own farms, and made mainly in the farmhouses.

Changes since the beginning of the century

Production of milk. During the last 50 years this zonal arrangement has almost disappeared. Now the sale of milk off the farms is almost universal. Even the remote farms have been enabled to sell milk by: (1) cleaner methods on the farm; (2) milk cooling on the farm; (3) motor transport for conveying milk from the farm to the depot or factory; (4) pasteurizing and efficient cooling in depots and factories.

The farmhouse processing of milk has dwindled to insignificant amounts, and manufacture of both cheese and butter is now mainly confined to factories. Tables 1 and 2 indicate some of these changes during the recent war period. These are still more striking if one puts them against the background of 1900 or 1908.

Table 1. *Production of milk in the United Kingdom**

	Average, 1936-9	1940-1	1943-4	1945-6
Cows and heifers in milk (thousands)	3283	3418	3576	3534
Average gross yield (gal./cow)	542	470	479	506†
	Gal. $\times 10^6$			
Gross production	1781	1608	1712	1789
Total available for human consumption	1563	1446	1580	1654
Total consumed as liquid	1002	1137	1339	1432

* Ministry of Agriculture and Fisheries, Department of Agriculture for Scotland and Ministry of Agriculture, Northern Ireland (1949).

† Edwards (1950) estimated that in 1948-9 the average yield of the 2,800,000 cows in England and Wales was 600 gal.

Table 2. *Utilization of milk in the United Kingdom**

	Average, 1936-9	1940-1	1943-4	1945-6
Gross production (gal. $\times 10^6$)	1781	1608	1712	1789
Total available for human consumption (gal. $\times 10^6$)	1563	1446	1580	1654
Total consumed as liquid milk (gal. $\times 10^6$)	1002	1137	1339	1432
Total made into cheese on farms (gal. $\times 10^6$)	34	15	6	5
Total made into butter on farms (gal. $\times 10^6$)	151	79	49	50
Total made into cream on farms (gal. $\times 10^6$)	18	5	—	—
Manufactured off farms (gal. $\times 10^6$)	358	210	186	167
Butter produced (tons $\times 10^3$)	46	26	18	17
Cheese (tons $\times 10^3$)	44	33	21	25

* Ministry of Agriculture and Fisheries, Department of Agriculture for Scotland and Ministry of Agriculture, Northern Ireland (1949).

Value of agricultural output. Table 3 summarizes the chief changes in the values of agricultural output during the last 40 years. Milk and dairy produce in 1908 provided 20 % of the British farm income. Now the proportion out of a greatly increased total is over 30 %.

Stock. The following points are brought out in Table 4: (a) The steady increase in the numbers of cattle, even in the prewar period. (b) Cows and heifers in milk or in calf have increased to a proportionately greater extent than the total cattle population. In 1894, they constituted 38.8 % of the total cattle population, in 1939, 44.5 %, and in 1946, 46.8 %. This in turn reflects the tendency towards milk production.

Table 3. *Changes in percentage value of different items of agricultural output in Great Britain in the last 40 years*

	1908*	1938†	1945†
All farm crops	30	16	23
Fruit, flowers, vegetables and nursery stock	5.5	14	20.5
Total livestock and produce	64.5	70	56.5
Dairy produce	20	26	31
Poultry and eggs	3.5	10	6.7
Other livestock and produce	41	34	18.8
Total value (£'s sterling × 10 ⁶)	151	265	546

* Board of Agriculture (1912).

† Compiled from statistics of the Ministry of Agriculture and Fisheries and the Department of Agriculture for Scotland (see, for example, Department of Agriculture for Scotland (1948, 1950); Ministry of Agriculture and Fisheries (1941); Ministry of Agriculture and Fisheries, Department of Agriculture for Scotland and Ministry of Agriculture, Northern Ireland (1948)).

Table 4. *Numbers of stock in Great Britain*

(Thousands)

	1894*	1939†	1946†	Loss or gain 1939-1946 (%)
Total cattle and calves	6,346	8,119	8,716	+ 7.3
Cows and heifers in calf or in milk	2,460	3,615	4,066	+ 12.5
Total sheep and lambs	25,800	25,993	19,718	- 24.1
Breeding ewes	—	19,572	8,018	- 24.2
Total pigs	2,390	3,767	1,643	- 56.4
Total poultry	—	64,137	47,276	- 26.3
Agricultural horses	1,000	649	519	- 20.0

* Board of Agriculture (1895).

† Ministry of Agriculture and Fisheries, Department of Agriculture for Scotland and Ministry of Agriculture, Northern Ireland (1950).

Table 5 is an attempt to indicate changes that have taken place in the relative numerical importance of a few of our British breeds. The first column gives the estimate prepared on the returns of a census made in 1908. Obviously, it can only be taken as a rough guide because, for instance, little or no account is taken of cross-breeds. Such a census has not been made since, but for recent years the numbers of bulls licensed afford a means of making a rough comparison. The figures should be taken with a good deal of reserve as a means of assessing the numbers of the different breeds, because the bulls of some breeds are used more for crossing than others. Still there can be no doubt that they do afford a reasonably true picture of the general changes. The chief of these are: (1) Increased importance of Friesians and Ayrshires. (2) Substantial reduction in the beef breeds. (3) Reduced importance of the dual-purpose breeds, though these, owing to the dominant position occupied by the Shorthorns*, still account for nearly half the bulls licensed in the country. Edwards (1950) estimated that in England and Wales in 1948-9 the cow population (2,800,000) included 34 % Shorthorns, 32 % Friesian, 11 % Ayrshire, 9 % Channel Islands, 14 % all others.

* All Shorthorns are grouped together, but, even if beef Shorthorns could be excluded, the percentages would not be greatly altered, e.g. all the Shorthorn bulls licensed in Scotland in 1945-6 do not amount to more than 1½ % of the total number of bulls licensed in Great Britain.

Table 5. *Changes in numerical importance of some British breeds of cattle*

Breed	Census of cattle, 1908*		Bulls licensed in Great Britain			
	No.	Percentage of total	1937-8†		1945-6†	
			No.	Percentage of total	No.	Percentage of total
Shorthorn	4,413,000	63.9	25,068	56.1	15,415	38.1
Devon	455,000	6.6	1,091	2.4	682	1.7
Ayrshire	440,000	6.4	5,083	11.4	7,203	17.8
Hereford	385,000	5.6	2,116	4.7	1,742	4.3
Welsh	248,000	3.6	349	0.8	401	1.0
Aberdeen Angus	194,000	2.8	2,530	5.7	1,533	3.8
Lincoln Red	169,000	2.4	1,324	3.0	1,242	3.1
West Highland	100,000	1.4	60	0.1	74	0.2
Channel Islands	101,000	1.5	2,496	5.6	1,850	4.6
Galloway	31,000	0.4	337	0.8	301	0.7
Red Poll	27,000	0.4	569	1.3	474	1.2
Friesian	—	—	2,914	6.5	8,565	21.2
Other breeds and descriptions	342,000	5.0	731	1.6	878	2.2
Total	6,905,000		44,668		40,360	

* Board of Agriculture (1912).

† Compiled from statistics of the Ministry of Agriculture and Fisheries and the Department of Agriculture for Scotland.

Table 6 gives some indication of the effect that changes in relative numbers of the different breeds may have on the milk supply. Apart from changes in relative numbers of breeds to which I have called attention, the numbers of cows now producing milk

Table 6. *Yields of milk per lactation of cows and heifers of different breeds in England and Wales*

Breed	No. of herds, 1948-9	Average yield, 1946-9* (lb.)
Ayrshire	2601	7374
Friesian	4752	8278
Guernsey	1393	6872
Jersey	1082	6448
Red Poll	425	6861
Shorthorn	5022	6660

* Milk Marketing Board (undated).

for sale have been greatly increased by the fact that many farms previously regarded as unsuitable for milk production have been tempted by the relatively high price of milk to embark on milk selling. Even in eastern counties, from Aberdeen to Norfolk, previously regarded as definitely wedded to arable farming, and where milk selling used to be regarded as hardly a respectable business, many herds of dairy cows have taken the place of fattening bullocks and flocks of sheep. But probably a still greater effect on the milk market has been produced in the western areas which always had cows of one kind or another, but did not think of selling milk. This applies especially to some of the poorer hill districts, where the traditional type of farming was associated with the rearing of store cattle and the keeping of sheep. The yields obtained on such

farms even from dairy breeds are much below those from herds in districts well suited for milk production, and where the business is well understood.

Management. Formerly the great majority of cows calved in spring, and thus by far the greater part of our milk supply was produced in summer. For instance, in 1878 a writer estimated that 76 % of cows in Great Britain calved between January and June; only 24 % between July and December. To a great extent this was arranged deliberately in order that full advantage might be taken of the summer growth of grass, but it was—and still is—also easier to arrange for spring calvings than for autumn calvings. The proportion now calving in autumn is very much higher than the figures I have just quoted. Even since the beginning of the war, the tendency for more autumn calvings has become increasingly obvious. For instance, in Cheshire, a county with a strong tradition for spring calving, because of its old cheese-making practice, the proportion of the annual output of milk in winter is now nearly 50 %. In the same county, the calves born from September to February inclusive, in 1949–50 came to no less than 66 % of the total cow population. It is well known that, other things being equal, cows calving in autumn or midwinter give a greater total yield of milk than cows calving in spring or summer, and this change in the distribution of calvings during the seasons has undoubtedly contributed to the great increase in milk production.

Concentrated foods. An adverse factor is the great reduction in the quantity of concentrated feeding-stuffs available, especially since the beginning of the last war. At that time we were importing as much as 8 million tons of feeding-stuffs. In addition, there were more by-products from the milling industry than now, because the extraction of flour in the milling process was lower. It was possible to use high-yielding cows largely as converters of cheap imported foods into milk. The outbreak of war changed that, and we see the effect in the reduction of milk output during the 1st or 2nd year of the war. Since then, there has been a steady recovery, partly due to increasing efficiency in the growing of arable crops for milk production on the part of farmers who, before the war, had probably never ploughed land at all, but also to improvement in grassland management, and the substitution of new grass for a great deal of the poor old permanent pasture.

Another contributing factor that must be remembered when thinking of increased production from grassland is the fact that the sheep formerly kept for fat lamb production on a great deal of grassland of the country were drastically reduced in numbers at the outbreak of war. Although they have increased again to some extent, the competition of the dairy cow has prevented return to anything like full prewar numbers. The effect of a flock of sheep on milk production can only be fully appreciated by those who have had to try to combine the two kinds of stock.

Although the quantity of concentrated food available is only a fraction of what was formerly fed to dairy cattle, there can be no doubt that far better use is made of the limited quantity available. The system whereby feeding-stuffs are rationed by the Ministry of Food, according to the quantity of milk produced in itself goes far to ensure efficient use. Moreover, apart from this official rationing, which determines the quantity of concentrates given to the whole herd on any particular farm, the great development of milk recording does much to ensure that having got to the farm the

food is distributed to the cows in the herd according to their individual production and needs.

Control of disease. Advances in control of disease have undoubtedly contributed very considerably to increased production. Tuberculosis, contagious abortion, milk fever, have within my own experience come largely under control. Mastitis may now almost be put on the same list, and sterility is being seriously attacked. All these diseases formerly took a heavy toll and caused direct loss of cattle, but perhaps even to a greater extent caused loss by reducing the efficiency of the cow as a milk producer. Even now, because of disease or sterility, large numbers of our dairy cows are sold for slaughter before they have passed the period of full efficiency, but the position is better than it was even 30 years ago. On the other hand, some people claim that the breeding and feeding of cows for high production necessarily reduces their ability to resist diseases, or to overcome the effect of minor disturbances. Within limits, which on the average we are not likely to pass, I do not think that there is any evidence to support this view, though it is well known that in some instances cattle that are kept on a low plane of nutrition, amounting to semi-starvation, such as the West Highland cattle in the Hebrides, are remarkable for their longevity.

Breeding. I have left to the last the effect of what we may term improvements in breeding. It is to the credit of the farmers of the south-west of Scotland, led by John Speir, that they were ahead of the rest of Great Britain in the establishment of the milk-recording movement. But though milk recording is the basis of constructive breeding of dairy cattle, it by no means follows that milk recording necessarily results in the rapid raising of production. So far as breeding is concerned, milk recording has mainly been used for (1) the selection of bulls on the performance of their dams and grand dams, (2) the culling of low-yielding cows. Even where it is applied in the most efficient manner by the selection and use of progeny-tested bulls, improvement is far slower than many people suppose. As some of my colleagues have shown in a series of papers, culling of low-producing cows in any herd by itself is only likely to effect very slow improvement indeed. A pedigree herd breeding its own bulls might make progress at a greater rate, but the greatest possibilities lie in artificial insemination units, breeding their own bulls from sires that have been progeny-tested and from a small percentage of the best cows. Such a unit, operating under ideal conditions, might improve the average yield at the rate of about 15 gal./year. This is very much below the expectations of many enthusiasts, but it is greater than the average improvement that has actually been obtained so far at artificial insemination centres. Hitherto, they have only been able to secure a very small proportion of progeny-tested bulls. Without going far into a genetical discussion, in which I should soon be far out of my depth, I may say that the fundamental difficulty about improvement by breeding is the low heritability of milk yield. If two cows in the same herd differ by about 100 gal. milk/year, their daughters are likely to differ by only about 12½ gal.

Of the 100 gal. difference between the two cows, only 25 gal. are due to genetic causes, which can be passed on to the progeny. The remaining 75 gal. difference is due to environmental factors, both during the calf's life, and also in its prenatal existence. Half the calf's genetic make-up comes from its sire, the other half from the dam, and

the 12½ gal. which I have mentioned represents 25 divided by 2. I am confident that taking herds of the same dairy or dual-purpose breeds, there has been improvement in milk yields brought about by the skill of the breeder aided by milk recording, but it is, I think, quite certain that the increase in milk yields that has taken place in perhaps the majority of well-managed herds, during the last 50 or even the last 20 years, has mainly been due to the other causes I have mentioned. I am, nevertheless, hopeful that in the course of the next 50 years, improvements in the selection of breeding stock, especially bulls, and the methods of using them will exert a much greater proportional effect than breeders have been able to secure in the past.

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Culinary Uses of Milk

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A pint of average milk supplies about 340 Cal., 18 g. protein, 680 mg. calcium, 0.6 mg. iron, 600 i.u. vitamin A, 0.2 mg. aneurin, 0.8 mg. riboflavin, 0.48 mg. nicotinic acid, 6 mg. ascorbic acid, and 12 i.u. vitamin D. The proportion this represents of our daily requirement is shown in Fig. 1. A pint of milk would therefore be an excellent addition to our diet for protein, calcium, vitamin A (summer only) and riboflavin. It is, on the other hand, poor in calories, in iron, and in nicotinic acid. The amount of ascorbic acid is variable and depends on the amount of oxidation. Calories, iron and aneurin are easily supplied at the present time by bread. Nicotinic acid is more difficult to obtain in adequate amounts during the present shortage of meat, although fish is a good source, and potatoes are a better source per 100 Cal. than is bread.

Le Gros Clark (1947) showed that the expectation of life is highest if the proportion of calories derived from bread and potatoes is less than 50 %. Cuthbertson (1942), in his work on wound healing, showed the value of animal protein, and concluded that the virile races of the world are the animal-protein eaters (Cuthbertson, 1950). We must, therefore, aim at keeping up our animal-protein intake. The lowest minimum adult requirement is 25 g., and the aim of this paper is to show how this value can be maintained by using raw or processed milks.

As a source of animal protein, milk is not expensive (Fig. 2). The standard used for meat in constructing Fig. 2 was stewing steak. Although meat is cheaper at the present controlled price, eggs are more expensive than milk and cheese, and fish and bacon

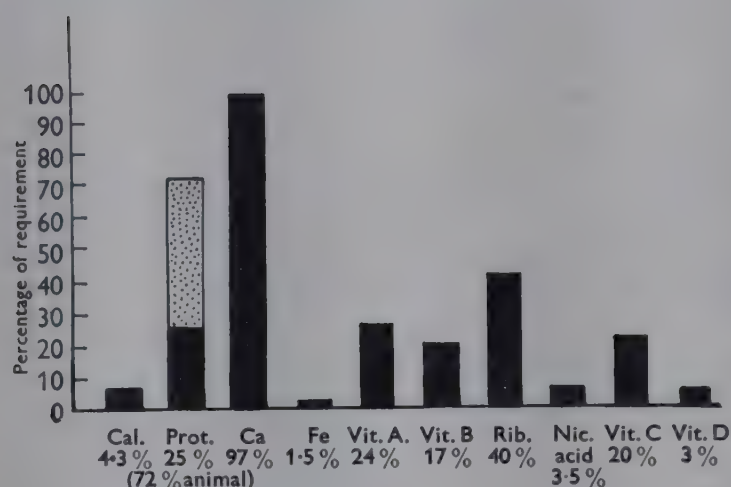


Fig. 1

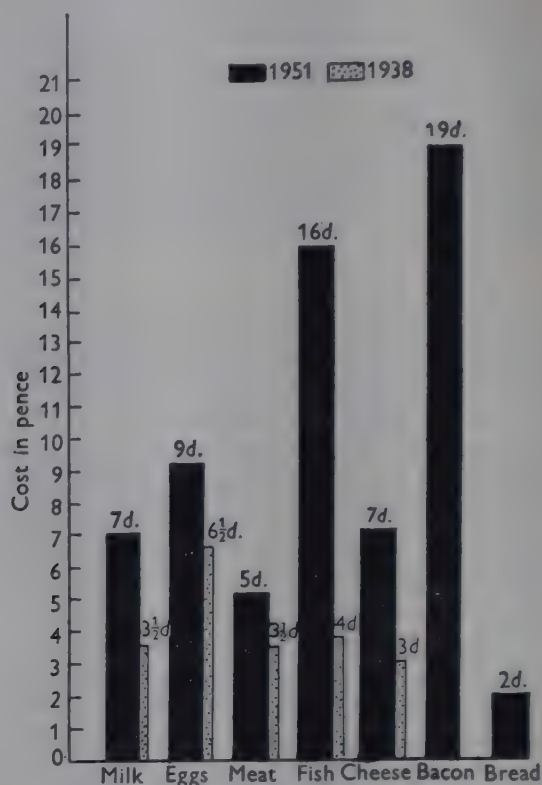


Fig. 2

Fig. 1. Percentage of daily requirement of nutrients supplied by 1 pt. of milk.

Fig. 2. Cost of 25 g. animal protein as milk, eggs, meat, fish, cheese and bacon.

are very dear. The present cost of bread protein is shown for comparison. Rabbit at 6s./lb. makes our protein requirement cost 3s. 6d. and chicken at 12s. 6d./lb. gives it the prohibitive value of 7s. 10d. It is interesting to compare the cost of protein requirement in 1951 with the cost in 1938. Bacon, unfortunately, was not included in our 1938 survey (Andross, 1941), but it can be seen that the cost of fish is treble, and that of cheese and milk double, the 1938 value. Meat and eggs have increased by less than one-third, presumably because the price is subject to control (Andross, 1941).

But rationing also makes us depend on milk. It is shown in Fig. 3 that we require about $\frac{3}{4}$ pt. to satisfy our animal-protein requirement, $\frac{2}{3}$ pt. to satisfy our calcium requirement, and 1 pt. to satisfy our riboflavin requirement, despite our allowances of other protein foods. The further aim of this paper is to determine how much of this quantity of milk can be used in cooking. Many adults do not drink milk, and still persist in regarding it as a beverage rather than as a food.

Milk in cooking

Milk in cooking is used in baking, in making white and cheese sauces, in the preparation of pouring and baked custards, milk puddings and cream soups. It is also used in preparing curds or junkets, milk jellies and ice-creams. In Fig. 4 the quantity of milk included in one helping of some of these products is shown, and compared

with that consumed in beverages. Incorporation of milk in cooked products is shown to be limited. Rice pudding and other milky puddings, baked custards, cheese sauce and creamed soups add considerably to the animal-protein content of the diet.

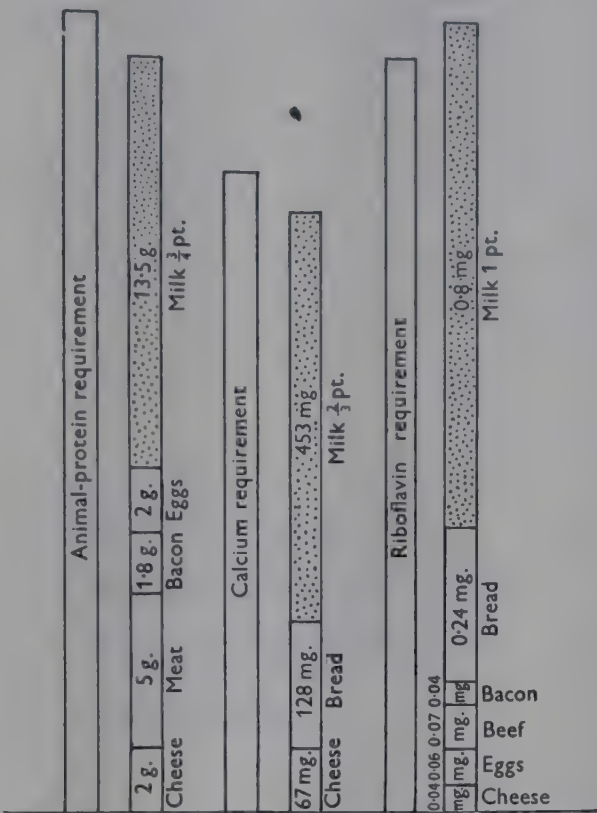


Fig. 3. Proportion of daily requirement of protein, calcium and riboflavin supplied by rations, and amount of milk which must be consumed to make up requirement.

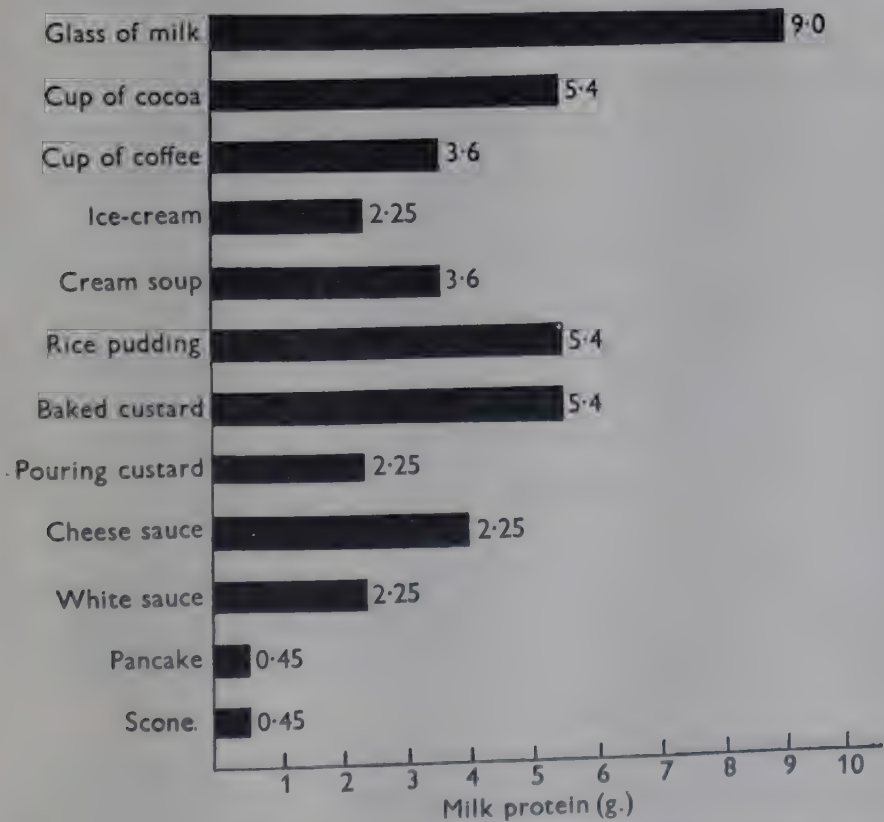


Fig. 4. Amount of milk protein in a helping of various dishes.

Otherwise the amount of milk that can be used in cooking seems somewhat low, especially in baked products. Nevertheless, the greatest care must be taken in altering standard recipes since the product must have its normal appearance and flavour, for bizarre combinations and unfamiliar types of food are almost certain to be rejected. The easiest way of increasing the milk intake is by the consumption of beverages that are normally taken once or twice daily.

The addition of milk to soup is sometimes followed by curdling and spoilage; this is particularly marked with tomato soup. Our results have not always been consistent, but we have found that it is safer to add cold milk to hot soup. Milk is altered by heating so that it curdles more easily and its pH is lowered by acid production, particularly if the milk is not fresh. Addition of a trace of sodium bicarbonate will prevent the curd. Slow heating in a double boiler also tends towards curdling. The isoelectric point for casein is at pH 4·6, and this is the point at which curdling occurs in a soup. If sufficient acid is added to lower the pH below 4·6, the casein may form soluble salts so that the curd disappears. This explains the preparation of uncurdled milk shakes with fruit juices. We have found that the safest way of making tomato soup is to prepare a sauce with flour and butter or margarine and either milk or tomato puree, and then to add the other ingredients. The flour forms an additive colloid with the casein and curdling does not take place.

Fish and vegetables can be baked in milk. Again the product can be spoilt by curdling which, however, can be prevented by addition of sodium bicarbonate. Salt fish is more likely to cause curdling than fresh fish. A favourite Scots dish is findon haddock poached in milk. We have found that the best way to prevent curdling in such fish dishes is to add the milk gradually during cooking. This prevents the precipitation of casein by overheating and decreases the amount of lactose caramelized. The amount of milk protein for one person from such a dish is 2·25 g. per helping.

Some vegetables always cause coagulation and therefore cannot be cooked in milk. Peas, carrots, beans and asparagus are examples. Cauliflower, cabbage and spinach do not cause coagulation. Although some vegetables are slightly acid the precipitation in this instance is thought to be due to the tannin and salt content rather than to the acidity. The tannin causes denaturation of the colloidal calcium caseinate and renders it more sensitive to the ions in the vegetable juices (Lowe, 1932).

Another method of using milk in cooking is in junkets in which milk is coagulated with rennin. Flavoured junket powders are now available, and junkets can also be sprinkled with chocolate or spice. From a nutritive point of view junkets have the same value as milk. A helping would be equivalent to from 1 gill to $\frac{1}{2}$ pt. of milk, i.e. 4·5–9 g. protein.

Milk jellies are made by setting milk with flavoured gelatin. Care must be taken to cool the gelatin after dissolving it and before adding the milk, otherwise the milk will curdle. Setting in a refrigerator is an advantage. If solid gelatin is used it must be swollen first in cold water; hot water is then added.

In the preparation of pouring custard in a double boiler, quick-boiling water in the outer jacket gives a thickening point at 87° and curdling at 90°. Slow-boiling water gives a thickening point at 82° and curdling at 87°. The interval between thickening

and curdling is, therefore, greater with slow boiling, which allows the cook a greater margin of safety.

Baking custards show a fall in temperature just before setting. The setting temperature varies with the recipe but a mixture consisting of one egg, one cup of milk and one tablespoonful of sugar will set at 87°. The only method of obtaining such a low temperature in an oven is to cook the mixture in a vessel contained in a basin of water (Andross, 1940).

Evaporated milks. Processed milks can be used to advantage in cooking. Excellent dishes can be made with condensed milk. This product contains 40 % sugar so no sweetening is required. Lemon-meringue pie can be filled with a tin of sweetened condensed milk, two eggs and the juice and rind of two lemons instead of the usual

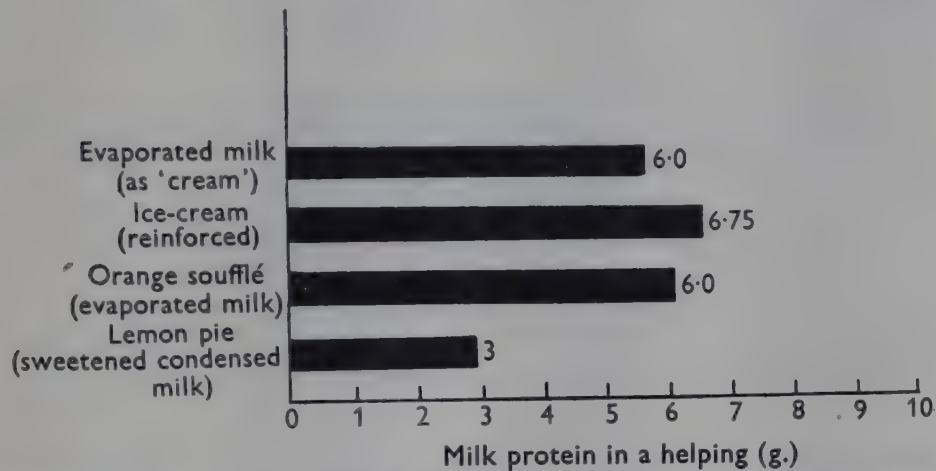


Fig. 5. Some ways of using evaporated milk and sweetened condensed milk to increase protein intake.

cornflour filling. Such a pie will supply six persons, and provide them with 3 g. protein each in addition to that in the normal recipe. A reinforced ice-cream for invalids can be made by freezing a tin of sweetened condensed milk and a tin of evaporated milk. A helping would supply the patient with 6.7 g. protein. Serving unsweetened evaporated milk with sweets is a good way of using evaporated milk. This will be more favoured in Scotland where it is customary to serve milk or cream with desserts. Half a gill can be consumed in this way, equivalent to 6 g. protein. Unsweetened condensed milk makes excellent cold soufflés. No egg is required. One tin of evaporated milk beaten until it is just setting with an orange jelly dissolved in orange juice sets in half an hour. This serves six persons giving each 6 g. additional protein (Fig. 5).

Dried milks. We have also experimented with dried milk, both skim and full cream, in order to find how much of each could be added to established recipes to increase the protein value. Scones, cakes and sauces were tested. Jack & Haynes (1951) reported a preference among boys for bread containing up to 14 % milk solids. This meant an increase in protein intake of 8 g. daily.

In our first experiments we used dried milk in place of raw milk in making scones. Penetrometer tests were made for texture, and density tests for lightness. A flavour score was also taken. The results, which are shown in Table 1, indicate that there was little difference in the product when dried milk was used instead of raw milk in scone

baking. They also showed that there was little or no advantage in reconstituting the dried milk before using it. In fact, unreconstituted milk mixed dry with the flour gave a lighter scone with a higher flavour score than reconstituted milk. The texture of the scones appeared to be slightly better with raw milk.

Table 1. *The effect of using dried milk (as a powder and also when reconstituted) as a substitute for raw milk in the baking of scones*

Type of milk used	Texture and density of scones		
	Penetrometer measurement (cm.)	Density (g./ml.)	Flavour score
Normal (raw)	6.7	0.012	1
Reconstituted dried	6.5	0.020	2
Dried mixed with flour; water added	6.6	0.011	1

Table 2. *The effect of increasing the proportion of dried milk used in the baking of scones*

Amount of dried milk added (g./½ lb. flour)	Texture and density of scones		
	Penetrometer measurement (cm.)	Density (g./ml.)	Flavour score
0	0.70	0.012	1
3.5	0.55	0.014	2
5.25	0.55	0.016	2
7.0	0.50	0.020	4

In our next set of experiments, 3.5 g. (one teaspoonful), 5.25 g. (one and one-half teaspoonfuls) and 7 g. (two teaspoonfuls) of dried milk were added to the normal scone mixture using ½ lb. flour. The same tests were performed as in the first set and the results are shown in Table 2. The scones became progressively denser as the proportion of dried milk was increased. Texture was only slightly affected. A high flavour score went to the mixture containing 5.25 g. dried milk because the high protein content gave the scones an attractive brown colour. The addition of 7.0 g. dried milk would be tolerated, but a greater quantity was detectable in flavour and was therefore disliked; also the scones felt very heavy. Our conclusion from these experiments is that 2.5 g. protein as dried milk can be added to scones containing 3.6 g. protein from raw milk, and the product still remain acceptable to the consumer. This is an increase in the content of milk protein of 69 %.

Cakes were also tested. Although it is generally accepted by experts that the addition of milk spoils a sponge cake, butter sponge was made containing three eggs, 6 oz. flour and 6 oz. sugar. If dried egg was used, 3.5 g. full cream, and 7 g. skim milk powder could be added without causing spoilage. If whole egg were used, double these quantities could be added. The penetrometer value fell as the amount of dried milk increased in the cakes. From these results it can be concluded that dried milk can be added to cakes in amounts that will increase the animal-protein value by about 10 %.

The viscosity in sauces was found to increase with the amounts of dried milk added,

but the amount of flour could be reduced to give the proper texture. A distinct preference in flavour score seemed to be for the sauce to which 3.5 g. of dried milk had been added per $\frac{1}{2}$ pt. raw milk. This is an increase in animal-protein content of 13 % (Fig. 6).

With rice puddings we found that 7 g. dried milk could be added per pint of milk without spoiling the flavour score. The best flavour score was made by an 11 % increase in animal protein (Fig. 6). It is interesting to compare these results with the experiments of Morse, Davis & Jack (1950) who found it possible to add double this amount to white sauces. Their results for cakes were also higher.

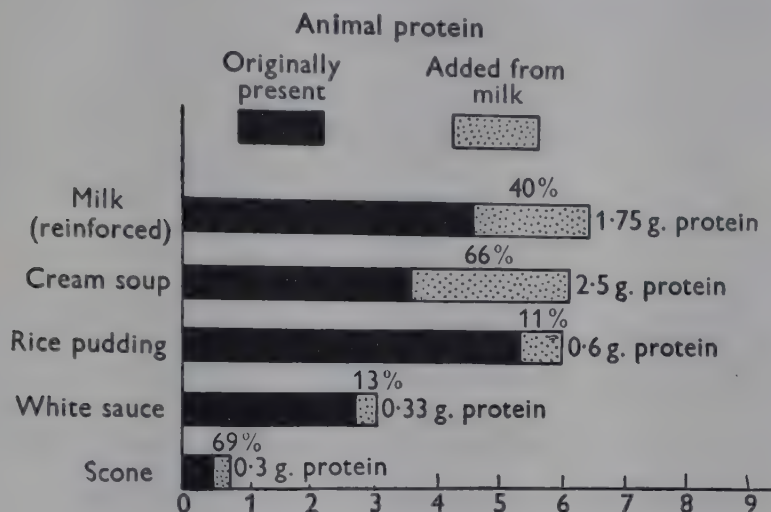


Fig. 6. Amounts by which intake of animal protein from a helping can be improved by adding dried milk to various dishes.

It is said to be possible to increase the protein content of a soup to a protein content of 9 g. per helping by the addition of dried milk (Fig. 6). This high value was not found acceptable by our tasting panel. They discarded soups having more than 6 g./0.5 pt. or helping. This is a 66 % increase in animal protein, and is equivalent to 7 g., or two teaspoonfuls, of dried milk. This would seem to be a large amount per helping, but the full flavour of the soup masks the flavour of the milk. Only cream soups with a strong flavour like celery will carry this amount.

Dried milk can be used in making icing, 'whipped cream' fillings for cakes, chocolate trinitads and the like, all of which increase the protein content of the diet. Care must be taken to use such uncooked products without delay, since dried milk is not bacteriologically sterile, and the bacteria in it will multiply in the presence of water unless they are killed by cooking.

One ice-cream recipe in common use employs 7 g. dried milk per helping. It contains, therefore, 2.5 g. animal protein. This is also the animal-protein value of ice-cream from raw milk. We found the texture unsatisfactory when we attempted to increase the proportion of dried milk. Dried milk can also be used in hospital diets where reinforcement of the diet is necessary by high-protein feeding. Such diets are now used in treatment of shock following accidents, burns and serious operations. They are also used in treatment of patients acutely ill with fevers, where consumption of food is greatly curtailed (Simmonds, 1948). The proportions in which dried milk is

used in such diets is 2 oz. dried full cream/40 oz. milk. Where the diet must be low in fat, 8 oz. dried skim milk are added to 40 oz. water.

Loss of nutritive value

The losses in the nutritive value of milk as a result of different types of processing were studied by Kon (1945). His main findings are shown in Table 3. Unsweetened evaporated milk suffers the greatest loss because of the high temperature at which it is processed. Evaporated milks and dried milks are usually made in the summer at the

Table 3. *Typical losses sustained by milk when processed*

Type of milk	(Kon, 1945)		
	Loss in		
	Biological value of protein (%)	Vitamin C (%)	Vitamin B ₁ (%)
Spray dried	5	20	10
Roller dried	5	30	? 10
Condensed sweetened	—	15	5-10
Evaporated	Slightly decreased	60	30-50

peak periods of milk production when the cows are at pasture so they are often richer in vitamin A and vitamin D than raw milk. The nutritive value of lactalbumin may be diminished by heating, probably owing to changes taking place in the linkages between the amino-acids which render them less susceptible to enzymic hydrolysis (Davis, Rizzo & Smith, 1949). Just before milk reaches boiling-point it becomes creamy like a custard, owing to setting of the lactalbumin envelopes around the fat globules. Further heating causes these to burst, when portions of them fall to the bottom of the pan, and other portions float to the top of the milk as skin. The albumin on the bottom of the pan is in a cup formation visible to the naked eye. It is very apt to char and give the product a burnt taste. For this reason milk should always be heated in a double boiler. This albumin occludes some of the calcium which is therefore lost. If the skin is discarded, as it often is, fat, protein and calcium are also lost. Skin should therefore be consumed where possible, e.g. it should not be skimmed off a milk pudding and discarded. The creamiest milk puddings are those cooked in the oven where the milk has been kept just below boiling-point (Andross, 1939). Overcooking, particularly at a high temperature, causes caramelization of the lactose, and the product assumes a brownish grey colour. The lactic acid produced may cause curdling. Casein is precipitated by prolonged heating above boiling-point.

Cream. Cream is an emulsion of fat in water, for which caseinogen and calcium caseinogenate are said to be the main emulsifying agents, but lactalbumin is probably also concerned in this process. When cream is whipped a temporary froth is formed by the protein. The air in the froth is surrounded by semi-solid fat clusters. These air-in-fat clusters form a network which becomes more stable as the clusters touch each other. The best whip is obtained if the fat is in a semi-solid condition. If cream is boiled or cooled it will not whip because the protein has been coagulated or precipi-

tated. Cream with a fat content of less than 30 % will not whip to a permanent emulsion since skim milk will separate from it on standing. Newly separated or newly pasteurized cream will not whip unless it is allowed to stand for 3 hr. Cream where acidity has developed, as in risen cream, whips more easily. Raw cream is better for whipping than pasteurized cream but its bacterial content may be high. The best whipping temperature is 7–8°.

Cream contains vitamin A and vitamin D in greater concentration than does milk. It contains an easily assimilated fat and is a food of high caloric value. Because it is so appetizing, its psychological value in food preparation is very great and for the past 10 years British cooks have been attempting to prepare substitutes for cream. High-class cooking is impossible without it, but a small quantity of it goes a long way in making a dish look attractive. We could use dried skim milk in baking to make a small amount of cream available for decorative purposes. This would certainly raise the standard of cooking in the British Isles.

SUMMARY

Under present conditions the British people require 1 pt./day of milk to reach their requirement of 25 g. animal protein, 670 mg. calcium and 1.24 mg. riboflavin.

Methods of prevention of curdling in soups, and fish and vegetable dishes made with milk have been determined.

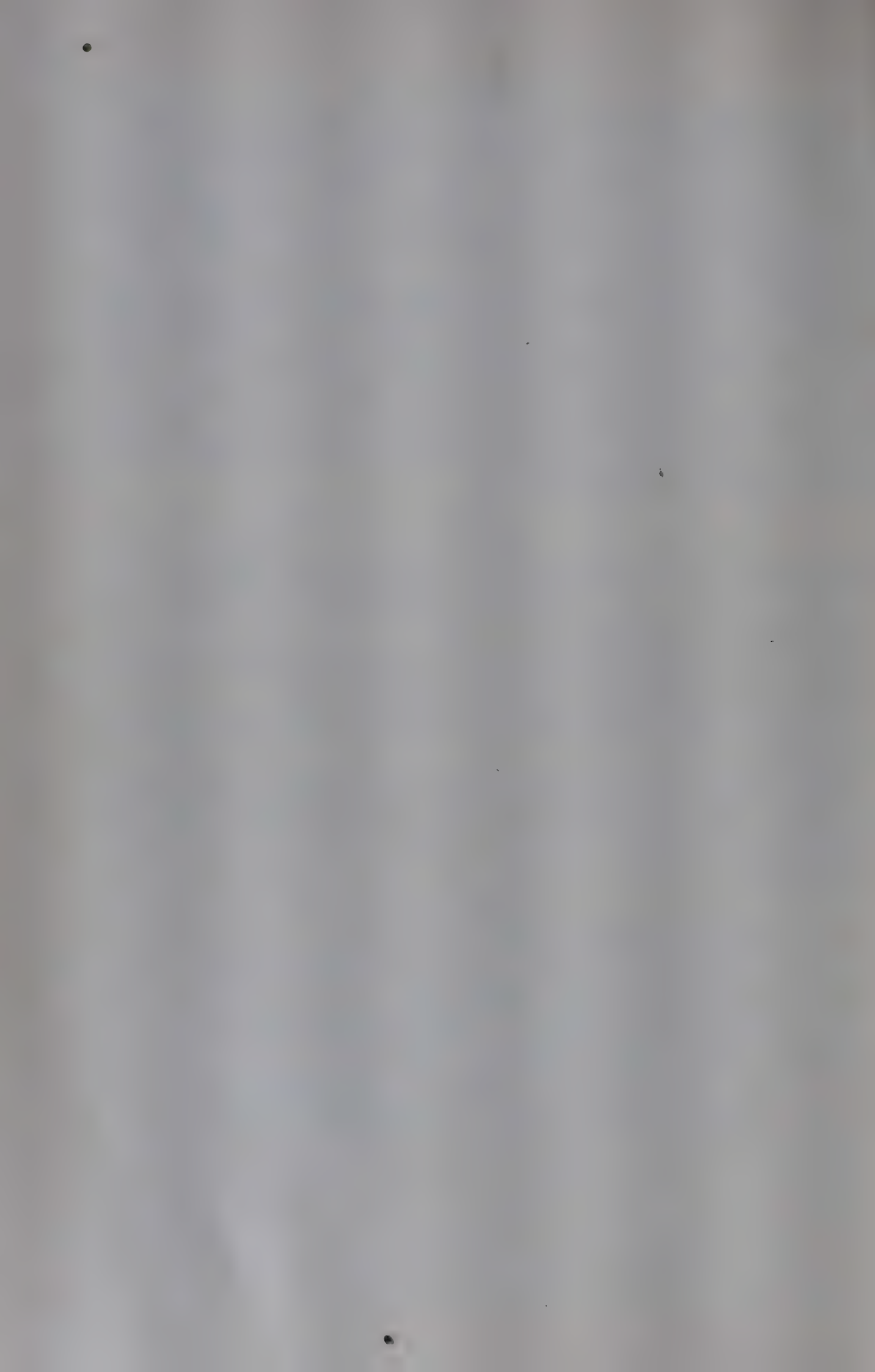
The amounts of animal protein provided by foods made with milk have been studied with particular reference to the contribution made by the milk.

The making of jellies and junkets has been investigated to find the best methods for their preparation.

Some methods of using condensed milks have been suggested, and the valuable amounts of animal protein they can contribute to the diet have been shown. The amounts of dried milk which can be added to certain products without spoilage have been determined.

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ABSTRACTS OF COMMUNICATIONS

The Sixty-fifth Meeting of The Nutrition Society (Thirtieth of the Scottish Group) was held at Strathcona House, Rowett Research Institute, Bucksburn, Aberdeenshire, on Saturday, 10 February 1951, at 2.15 p.m., when the following papers were read:

Effects of Cobalt Deficiency on Appetite. By J. STEWART, *Moredun Institute, Gilmerton, Edinburgh*

The Haemoglobin Concentration in the Blood of Male and Female Students.

By J. BECK and MARY WISHART (introduced by R. C. GARRY), *Institute of Physiology, University of Glasgow*

A marked difference between the haemoglobin concentrations in the blood of men and of women is accepted as 'normal'. Provided the environmental influences on the two sexes are similar then three possible explanations of this difference are (1) influence of an intrinsic endocrine factor; (2) a relatively greater plasma volume in the female; (3) inadequate iron intake by women to compensate for iron loss.

Table 1

Survey	Sex	No.	Mean Hb (g./100 ml.)	S.D.	S.E.M.
1949	M.	24	15.92	1.11	0.23
	F.	41	14.02	0.86	0.13
1950	M.	53	15.62	1.01	0.14
	F.	55	13.61	0.95	0.13

It is difficult to find groups of men and women which do not differ widely in their nutrition, environment and activity. In an attempt to eliminate such extrinsic influences, two surveys were carried out on men and women students at the same stage of their undergraduate career. They formed a 'population' where the environmental influences were practically identical. The majority were medical students in the 2nd year of their course, and their ages ranged from 18 to 23. The results are given in Table 1. The first survey was carried out in October–November 1949, the haemoglobin concentrations being determined visually by a Keeler grey wedge photometer. In the second survey in October–November 1950, the determinations were made photo-electrically with a Spekker absorptiometer.

The Stature of University Students and their Parents. By J. V. G. A. DURNIN and J. B. DE V. WEIR (introduced by R. C. GARRY), *Institute of Physiology, University of Glasgow*

At the last meeting of the Scottish Group of The Nutrition Society it was shown that present-day children are growing faster than those of earlier generations. A matter of some interest is whether as a result of their faster growth their adult stature is also increased or merely earlier attained. At the meeting, on the suggestion of Dr B. Woolf, the audience were asked if they were taller or shorter than their respective parents and a show of hands indicated that they were taller.

As a follow-up, the heights of students attending the Physiology class at Glasgow University were compared with those of their parents. The students (sixty-three females and 184 males) were measured in the Department and were given precise printed instructions for measuring their parents at home. The results showed that 76.2% of the women students were taller than their mothers and 73.9% of the men taller than their fathers. The differences of these percentages from the expected 50% on a null hypothesis are highly significant. The mean height of the women students was $64.37 \pm 0.25^*$ in. and of their mothers 62.73 ± 0.27 in., a difference of 1.64 in. The mean height of the men students was 69.08 ± 0.19 in. and of their fathers 67.31 ± 0.20 in., a difference of 1.77 in. It is difficult to accept with Morant (1950) that these differences are due to a real decrease in the heights of the parents.

REFERENCE

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* Standard error of the mean.

Experimental Muscular Dystrophy in Young Calves. By K. L. BLAXTER (in Receipt of a Senior Award of the Agricultural Research Council), P. S. WATTS* and W. A. WOOD, *The Hannah Dairy Research Institute, Kirkhill, Ayr*

Deaths in calves, 2-3 months old, due to heart failure and to muscular dystrophy have been studied. Four pairs of calves were given rations, containing dried skim milk powder, lard, and vitamins A and D in arachis oil. A further four pairs received identical diets, save that vitamins A and D were supplied as veterinary cod-liver oil. One animal of each of the eight pairs received 50 mg. racemic α -tocopheryl acetate daily. Dystrophy developed in those calves receiving the arachis oil diet without α -tocopherol, but not in those given this diet with the α -tocopherol. It occurred in both groups given cod-liver oil, the incidence being greater and the lesions more severe in those which did not receive α -tocopherol. Dystrophic musculature was associated with abnormalities of gait and behaviour. In mild cases body-weight gain and nitrogen metabolism were but slightly affected. In severe cases there was an elevation of the basal metabolic rate and an increase in the elimination of creatine in the urine. Brain oedema and slight gliosis of the spinal cord suggested a nervous involvement. Auscultation

* Now at the Institute of Veterinary and Medical Science, Adelaide.

tion and electrocardiography showed cardiac involvement. At post-mortem examination the dystrophy was found to be bilaterally symmetrical, and degeneration of muscle cells with proliferation of sarcolemmal elements was demonstrated histologically. Water, ash, fat, cholesterol, and distributions of nitrogen and phosphorus were determined in the muscle.

Observations on Diet during First Pregnancies in Aberdeen. By A. M. THOMSON, *Department of Obstetrics, University of Aberdeen*

Reproduction in the Mouse as Affected by Additions of Calcium Carbonate to the Diet. By MARION B. RICHARDS, *Rowett Research Institute, Bucksburn, Aberdeenshire*

In a breeding test on mice, using the method of continuous monogamous mating, three levels of calcium carbonate—0.5, 1.0 and 2.0%—were added to the B diet of Sherman and to three modifications of it. The Ca content of the basal diets was 0.34% and the Ca : P ratio approximately 0.7. On all the diets the highest addition of calcium carbonate, which gave a Ca intake of 1.1% and a Ca : P ratio of approximately 2.3, significantly lowered the number and total weight of young weaned, and increased the number and proportion of deaths. Post-mortem examination of the weanlings showed the presence of pale speckled livers, enlarged hearts and small thymus glands in the groups receiving the higher amounts of calcium carbonate.

When supplements of iron citrate, sodium acid phosphate or calcium dihydrogen phosphate were added to the diet, a number of failures to rear litters occurred in the high-Ca groups, but in the litters which were reared the weaning weight seemed to be favourably affected by addition of iron or by reduction of the Ca : P ratio. The heart weights of the weanlings were significantly increased by Ca and decreased by iron, while thymus weights were diminished by Ca and increased by iron.

The Blood and Liver of the Mouse as Affected by Additions of Calcium Carbonate to the Diet. By W. A. GREIG, *Rowett Research Institute, Bucksburn, Aberdeenshire*

Weanling litters from young adult female mice fed on modifications of the B diet of Sherman with an addition of up to 2% calcium carbonate exhibited marked anaemia; the mothers themselves also developed anaemia, but to a milder degree. The anaemia appeared to be of iron-deficiency type in every respect except that the mean cell volume was not clearly reduced; an attempt to explain this anomaly has been made on the basis of the peculiarly large number of immature red cells normally found in mouse blood. A remarkably close correlation was found to exist between blood haemoglobin and heart weight, especially in the weanlings. Other features noted in the weanlings included increased subcutaneous fatty deposits, hyperlipaemia, and marked fatty infiltration of the liver.

Further supplementation of the diet with ferric citrate partially or completely prevented these effects, but the addition of sodium acid phosphate did not do so to any significant extent; the addition of an equivalent amount of calcium as calcium dihydrogen phosphate, however, appeared to be less injurious than the addition of calcium carbonate.

Some Effects of Supplementary Feeding of Scottish Blackface Ewes and their Lambs. By J. W. HOWIE, *Rowett Research Institute, Bucksburn, Aberdeenshire*

The Use of Chromium Oxide to Measure the Apparent Digestibility of Carotene in Goats and Cows. By R. CHANDA, HELEN M. CLAPHAM, MARY L. MCNAUGHT and E. C. OWEN, *Hannah Dairy Research Institute, Kirkhill, Ayr*

The digestibility of carotene in dried grass was measured by the Cr_2O_3 method in both cows and goats. In the goats the direct method was simultaneously used for comparison with the Cr_2O_3 method. By the direct method digestibilities of carotene in four goats were 68.4, 63.0, 62.2 and 61.6%. The corresponding digestibilities obtained from the same faeces samples by the Cr_2O_3 method (Edin, 1918), after correction as suggested by Kreula (1947), were 67.4, 63.5, 62.4 and 61.4%. In six cows on a diet similar to that of the goats the Cr_2O_3 method showed 59.3, 54.0, 54.4, 54.4, 57.1 and 55.5% carotene to be apparently digested.

It was shown statistically that fewer animals for a longer time gave a more reliable result than more animals for a shorter time.

The use of the method to demonstrate the effects of thyroxine and thiouracil on the digestibility of carotene (Chanda, McNaught & Owen, 1951) was described. A detailed account of this work is in the press (Chanda, Clapham, McNaught & Owen (1951).

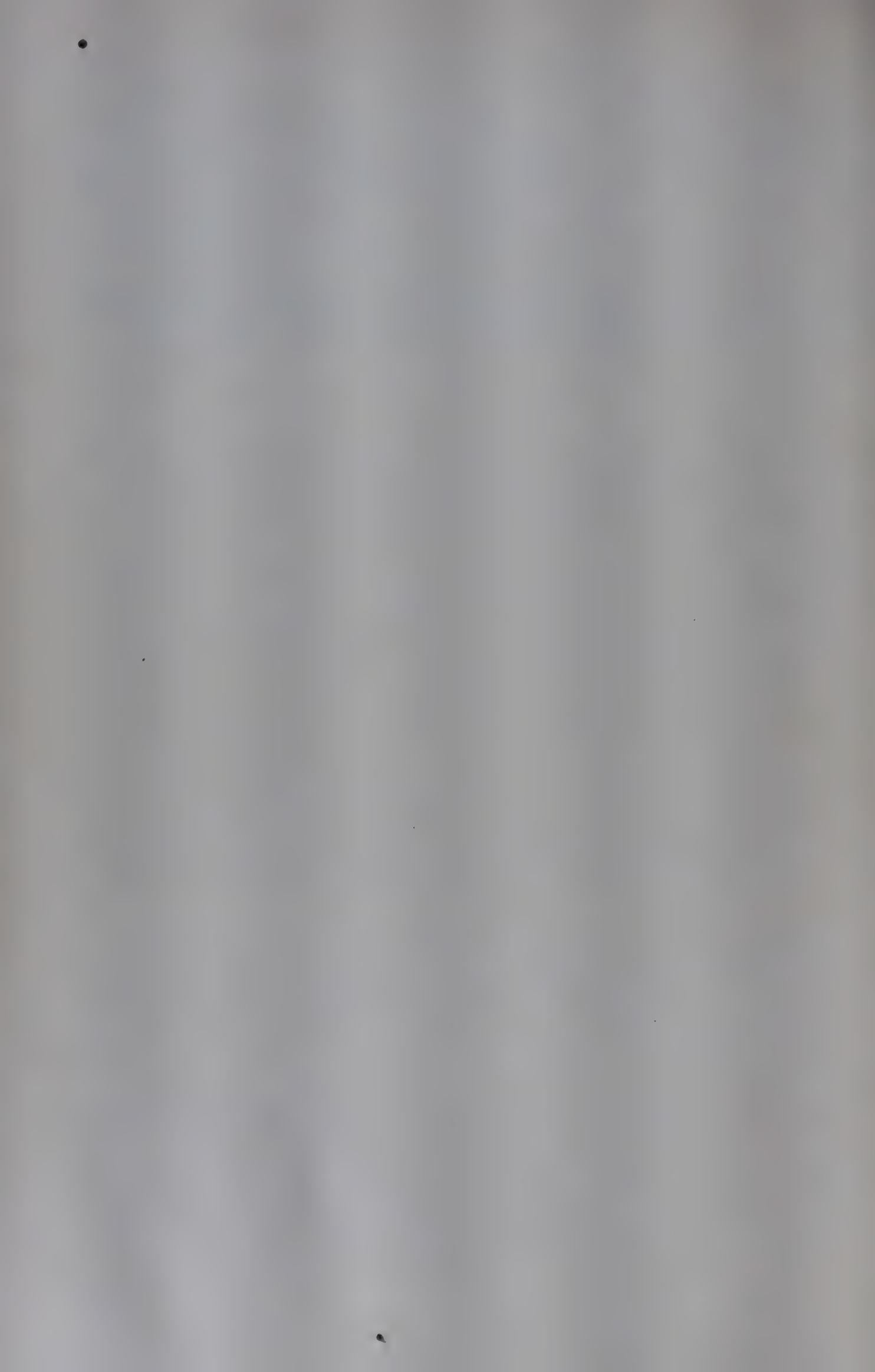
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The Response of Dairy Herds to a Single Dose of Copper. By G. DUNLOP, *Auchincruive, Ayr*

Some forty farmers are co-operating with The West of Scotland Agricultural College in an investigation on the nutritional adequacy of rations fed to dairy herds. Altogether 2500 cows are on experiment. Feeding-stuff firms, mineral-lick manufacturers and vitamin suppliers are assisting in the work.

Following the addition of 10 g. copper sulphate to an evening concentrate allowance 24 hr.—10 days before the visit of the milk recorder, positive responses in butterfat production have been obtained in herds fed rations supplied, or contributed to, by National feeding-stuff firms and mineral-lick manufacturers. Responses have been observed in certain cows in herds averaging over 4 % butterfat. The low fat percentage in the milk of certain animals in a herd exhibiting cows at the London Dairy Show has been shown to be due to deficiency of copper. No response has been elicited in less intensively farmed districts where the herds are fed the local feeding-stuff merchant's rations or the farmer's own dairy mixtures which do not include trace elements.



[The Editors of the British Journal of Nutrition accept no responsibility for the abstracts of papers read at ordinary scientific meetings of The Nutrition Society. These are published as received from the authors.]

ABSTRACTS OF COMMUNICATIONS

The Sixty-eighth Meeting of The Nutrition Society was held at the London School of Hygiene and Tropical Medicine, London, W.C. 1, on Saturday, 26 May 1951, at 10.30 a.m., when the following papers were read:

The Net Energy Value of Whole Milk as Determined by Respiration Calorimetry. By K. L. BLAXTER, *Hannah Dairy Research Institute, Kirkhill, Ayr*

A series of 24 hr. balances of carbon and nitrogen were made with an Ayrshire bull calf which received cow's milk as its sole diet. An open-circuit respiration chamber was used to determine respiratory carbon dioxide. Oxygen consumption and respiratory quotient were also determined. Experiments were made when the calf was given both 4 and 8 l. milk each day. It was found that activity of the calf within the confines of its cage was an important variable. The metabolizability of the gross energy of the milk was 95 % and the net availability of the metabolizable energy was 85 %. Net energy values calculated by the application of Rubner's factors for simple-stomached animals to the nutrients digested by the calf agreed with the experimentally determined net energy values. Kellner's factors which were determined with mature cattle, however, grossly underestimated the net energy value of milk in the young calf. The high heat increment of the adult ruminant compared with the low heat increment of the young calf supports the contention that the products of rumen fermentation enter the cycles of intermediary metabolism with a considerable thermodynamic loss.

Gustatory Enzymes. By A. F. BARADI and G. H. BOURNE, *Department of Histology, London Hospital Medical College, London, E. 1*

A number of enzymes have been shown by histochemical methods to be located in or around the taste buds in the papilla foliata of the rabbit. These include, alkaline and acid phosphatases, a simple esterase, lipase, muscle adenylase, and a nuclease. Alkaline phosphatase (and possibly other enzymes) is also associated with the gustatory organs in bat, monkey and man.

Various substances with pronounced taste (vanillin, quinine, peppermint, etc.) inhibited the activity of some gustatory enzymes and either had no effect on, or accelerated, the activity of others, e.g. vanillin inhibited phosphatase but not esterase or nuclease activity; quinine inhibited the esterase, did not affect the phosphatase, but accelerated the nuclease activity.

It is believed that the chemical mechanism of taste is associated with this process of differential enzyme inhibition or acceleration and that this mechanism is capable of distinguishing between an infinite number of tasting substances. It also explains why

substances of widely differing chemical structure can have a similar taste, since if they inhibit the same enzymes they will be responsible for identical impulses reaching the gustatory centre in the brain.

The same enzymes are present in the nasal mucosa, and it seems that the chemical mechanisms of both tasting and smelling are the same.

Antibiotics and Liver Extract for Suckling Pigs. By R. BRAUDE and K. G. MITCHELL, *National Institute for Research in Dairying, University of Reading*

Twenty-four blocks of six litter-mate pigs were dosed daily for 3 weeks during the 2nd to 5th week of life, with 10 mg. of either penicillin or streptomycin, with and without a liver extract. Control animals received liver extract only or no supplement. Twelve blocks were reared indoors and twelve on pasture. Both the meal mixture fed to the sows and the suckling-pig meal mixture fed in creeps contained fish meal.

At weaning, when 8 weeks old, the weights of the pigs indicated that there was no growth-promoting effect due to supplementations with penicillin or streptomycin. Previous findings (Braude, 1949) that pigs reared indoors benefit from an addition to their diet of a small quantity of liver extract, and that, irrespective of treatment, pigs reared out of doors grew better than those reared indoors have been confirmed.

We are most grateful to Miss P. M. Clarke for carrying out the statistical analysis on the data, and to Glaxo Laboratories Ltd. for the liver extract and the antibiotics.

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The Value of Antibiotics for Fattening Pigs. 1. As Supplements to Normal Fattening Rations. By R. BRAUDE, S. K. KON and K. G. MITCHELL, *National Institute for Research in Dairying, University of Reading*

The value of antibiotics and antibiotic residues as supplements to a basal fattening ration containing fish meal was tested in an individual feeding experiment involving nine replicates of five treatments, and five replicates of the sixth treatment. The experimental unit consisted of six litter-mates of similar initial weight. The experiment lasted 18 weeks starting when the pigs were 13–14 weeks old. The following six treatments were used: (1) control—basal meal only; (2) basal meal with 2 mg. aureomycin; (3) basal meal with 12 mg. penicillin; (4) basal meal with 62.5 mg. penicillin; (5) basal meal with 1814 mg. aureomycin mash (0.4 %); (6) basal meal with 1814 mg. streptomycin residue plus 12 mg. penicillin. All supplements are expressed per lb. of meal.

There were no significant differences between the treatments as far as rate of growth and efficiency of food utilization were concerned. There was, however, an indication that the pigs receiving aureomycin mash (treatment 5), aureomycin itself (treatment 2) and those on the higher level of penicillin (treatment 4) grew at a slightly greater rate than control pigs.

We are most grateful to Dr T. H. Jukes of Lederle Laboratories Inc. for the gift of the aureomycin preparations, and to Glaxo Laboratories Ltd. for the gift of the penicillin and streptomycin preparations.

The Importance to Sheep of Frequent Feeding. By J. G. GORDON (introduced by D. E. TRIBE), *Rowett Research Institute, Bucksburn, Aberdeenshire*

The Effects of Thyroxine and Deprivation of Carotene on the Secretion of Carotene and the Alcoholic Form of Vitamin A in Cow's Milk. By R. CHANDA and E. C. OWEN, *Hannah Dairy Research Institute, Kirkhill, Ayr*

Depriving cows of carotene by replacing a diet containing carotene by a comparable one not containing carotene, did not affect the yield of milk. Nevertheless, the carotene and vitamin A contents of the milk were diminished. On reinstatement of the carotene diet the carotene and vitamin A contents of the milk increased. The rates of these increases were accelerated when the reinstatement was accompanied by thyroxine treatment.

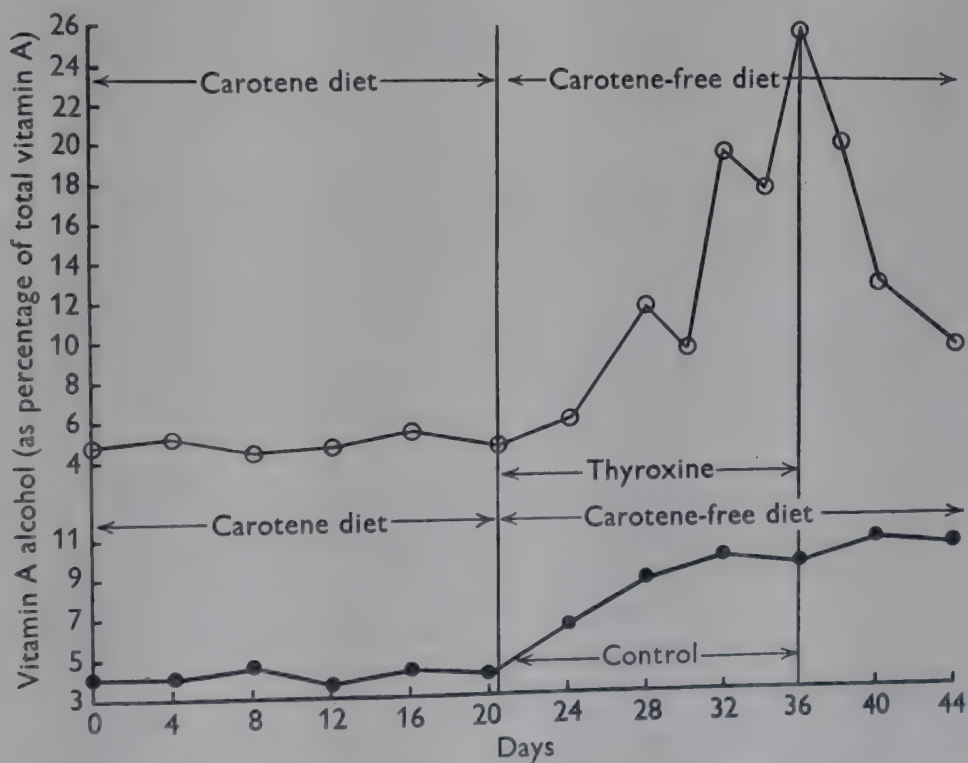


Fig. 1. The combined effect of thyroxine and deprivation of carotene on the secretion of vitamin A alcohol in the cow's milk.

On the carotene-free diet the percentage of vitamin A present in the alcoholic form increased. When thyroxine treatment was superimposed on a carotene-free diet the percentage of vitamin A in the alcoholic form showed dramatic increases corresponding to increases of total vitamin A. This effect of thyroxine on the milk of a cow deprived of carotene is shown in Fig. 1.

The Partition of Carotenoids and of Vitamin A in the Milk of Cows and Goats. By R. CHANDA and E. C. OWEN, *Hannah Dairy Research Institute, Kirkhill, Ayr*

When ingesting the same amount of carotene per unit body-weight, goats secreted more vitamin A in their milk than did cows. In the light of earlier observations of Chanda, Clapham, McNaught & Owen (1951*a, b*) this superiority of the goat over the cow in respect of yield of vitamin A in the milk per unit body-weight is attributable to the greater activity of the thyroid gland of the goat. Neither carotene nor vitamin A alcohol was measurable in goat's milk though both were present in cow's milk. β -Carotene was, however, found in goat's colostrum. Variable amounts of β -carotene were demonstrated in goat's liver but none could be found in the kidneys. Goat's liver contained amounts of vitamin A comparable with those reported for sheep by Moore & Payne (1942).

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Pathological Changes in the Rat in Deficiency of Essential Fatty Acids. By V. RAMALINGASWAMI and H. M. SINCLAIR, *Laboratory of Human Nutrition, University of Oxford*

During studies of skin changes in essential fatty-acid deficiency in the rat, the changes in several organs and blood were investigated. The deficiency syndrome was characterized by failure of growth, lesions of the skin and muco-cutaneous junctions and increased water consumption. The urinary output was not increased and there was no haematuria. There was also no evidence of fluid retention since the moisture content of various organs and of carcasses of deficient animals did not differ from that of the controls.

Changes in the lips and angles of the mouth, together with the well-known changes in the paws, were the first to appear at about the 6th week of deficiency and were similar to those observed in pyridoxin deficiency (Ramalingaswami & Sinclair, 1950*a*). As in pyridoxin deficiency (Ramalingaswami & Sinclair, 1950*b*) elevation of the erythrocyte count and reduction of mean corpuscular volume were found in essential fatty-acid deficiency but were much less severe. The liver, spleen, pancreas, kidneys, heart, thymus, stomach, small and large intestines, salivary glands, base of the tongue, Harderian glands, eyes and testes showed no definite microscopic abnormalities. In the lungs of the deficient rats, however, collections of large foamy phagocytic cells, resembling 'heart-failure cells', were found in groups of alveoli.

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The Relation of Deficiencies of Vitamin A and of Essential Fatty Acids to Follicular Hyperkeratosis in the Rat. By V. RAMALINGASWAMI and H. M. SINCLAIR, *Laboratory of Human Nutrition, University of Oxford*

In five experiments involving the use of 132 rats, the effects on cutaneous structure (anterior abdominal wall) of deficiencies of vitamin A and of essential fatty acids were studied. The criteria adopted for determining the identity of the experimental lesion with that in phrynoderma were plugging of the orifices of hair follicles with dense compact masses of keratin and acanthosis of the lining epithelium of these orifices, together with acanthosis and surface hyperkeratosis of the epidermis.

In deficiency of essential fatty acids, the changes were closely similar to those in phrynoderma, resulting in plugging of follicular openings with dense compact layers of keratin and acanthosis of their lining epithelium. In deficiency of vitamin A, however, they consisted of dilatation and loose hyperkeratosis of the upper third of hair follicles, with atrophy of their lining epithelium, and were similar to those described by Sullivan & Evans (1943) and Moulton (1943). This change could not be modified appreciably either by prolonging the period of deficiency by intermittent vitamin A supplementation, or by inducing partial deficiency states of vitamin A, or by using adolescent rats.

On the basis of this experimental evidence, it is suggested that deficiency of essential fatty acids may be the cause of phrynoderma in man. The clinical literature on phrynoderma, which was reviewed, is not incompatible with this hypothesis.

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The Content of Haemopoietic Factors in some Human Tissues. By R. H. GIRDWOOD, *Department of Medicine, University of Edinburgh*

In a patient with untreated pernicious anaemia who died of a coronary thrombosis, no vitamin B₁₂ was detected by microbiological assay using *Lactobacillus leichmannii* as test organism in the liver, kidney, spleen, lung, brain, stomach wall, intestinal wall, or muscle. Growth factors for *Streptococcus faecalis* (presumably chiefly pteroyl-glutamic acid, folinic acid, or both, or their conjugates) were present in all these tissues, chiefly in the liver and kidney.

Skin-biopsy specimens from three pernicious-anaemia patients in relapse contained growth factors for *Lb. leichmannii*, destroyed by alkaline hydrolysis (presumably vitamin B₁₂) amounting to 2.5 µg./100 g., 2.4 µg./100 g., and negligible quantities respectively. The first two amounts were comparable to what was present in control patients. Growth factors for *Strep. faecalis* were present in amounts comparable to those in the controls.

In a patient who died of primary malnutrition without anaemia, and who in the 24 hr. before death had not eaten foods containing significant amounts of vitamin B₁₂, there was a high bacterial count in the jejunum and ileum after death, with 7.8 µg. vitamin B₁₂/100 ml. of jejunal contents in the jejunum and 6.4 µg./100 ml. in the

ileum. The liver contained 38.8 μg . vitamin B_{12} /100 g., 446 μg . *Leuconostoc citrovorum* factor/100 g. and, in addition, 134 μg . pteroylglutamic acid/100 g. The kidney contained about half these amounts of the various factors. It appears possible that synthesis of vitamin B_{12} by intestinal organisms was important in this patient.

The Effect of Dietary Lactose on the Response of the Rat to Vitamin B_{12} .

By W. F. J. CUTHBERTSON and DOREEN M. THORNTON, *Research and Development Division, Glaxo Laboratories Ltd., Greenford, Middlesex*

An attempt has been made to develop a rat-assay method for vitamin B_{12} based on the observation that dietary lactose depresses the growth of rats (Ershoff, 1949) and that this effect may be overcome by vitamin B_{12} (Hartman, Dryden & Cary, 1949). A marked depression of growth was observed in weanling stock rats fed on a soya diet containing 20 % lactose. This effect was completely overcome by 30–40 μg . vitamin B_{12} (orally)/week. Rats from dams on soya-glucose (vitamin B_{12} -deficient) diet grew at still lower rates and 40 μg . vitamin B_{12} /week did not restore growth to normal.

The amounts of vitamin B_{12} required to produce significant growth increments were such as to make impracticable vitamin B_{12} assay based on its growth-promoting effects for these rats.

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Effect of Parental Nutrition on the Growth Response of the Rat to Vitamin B_{12} .

By W. F. J. CUTHBERTSON and DOREEN M. THORNTON, *Research and Development Division, Glaxo Laboratories Ltd., Greenford, Middlesex*

Vitamin B_{12} improves the growth of rats on vegetable diets (Emerson, Wurtz & Zanetti, 1949) especially if the animals are from dams on such diets (Emerson *et al.* 1949; Dryden, Hartman & Cary, 1949; Bosshardt, Paul, O'Doherty, Huff & Barnes, 1949). This effect of parental nutrition has been studied during development of an assay procedure for vitamin B_{12} on rats.

Animals were fed on a soya-glucose diet—the 'deficient' diet—from weaning. The response to vitamin B_{12} supplements was studied over the following 28 days. Stock rats grew well on this diet, vitamin B_{12} supplements having only slight beneficial effects. Rats from the first litters of dams given the deficient diet since mating did not grow well, but with supplements of 1–2 μg . B_{12} /week showed normal growth. Animals from the second and third litters grew as well as the first-litter animals on the soya-glucose diet, but higher supplements of vitamin B_{12} were required for maximal growth. Mortality was greater in the second and third litters.

Animals from the first litters of dams on the deficient diet were most suitable for vitamin B_{12} assay.

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